Monitoring river catchments in relation to potential environmental impacts from aquaculture activities

Trinh Thi Mong Tran (MSc, BSc)

Institute for Marine and Antarctic Studies

University of Tasmania

Submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

November, 2019

Declarations

Statement of originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Statement of authority of access

This thesis may be available for loan and limited copying in accordance with the *Copyright Act 1968*.

Signed: Date: 25th November 2019

Trinh Tran

Abstract

Numerous factors affect stream macroinvertebrate communities including river geomorphology, natural habitat within the catchment and human influences. In Tasmania Australia, a number of salmonid hatcheries discharge aquaculture effluent into adjacent rivers but the impacts of this waste water on macroinvertebrate communities of these receiving waters is not well known despite being essential for informing management options. In this thesis, I examined spatial and temporal patterns in macroinvertebrate communities in rivers with and without aquaculture farms from two regions of Tasmania (north and south) across multiple times to assess the evidence for impacts of farms on macroinvertebrate communities. I also assessed the impacts of a major flood on macroinvertebrate communities in rivers. I then explored the potential for this information to be used as a simple, quick and reliable monitoring tool for aquaculture farms wishing to manage and monitor waste discharge effluents on streams.

The first data chapter (Chapter 2), describes differences in macroinvertebrate communities in rivers without farms in the northern and southern regions of Tasmania and across time to assess background patterns of macroinvertebrate community structure in Tasmanian rivers. Macroinvertebrate community composition was not different between the two regions but differed significantly among rivers reflecting differences in stream geomorphology, natural habitat of the catchment, and biological conditions (source of pollution). Four upland rivers surrounded by forest all had a similar community structure which indicated good water quality while four lowland rivers surrounded by grazing and agriculture had a different community structure (but were similar to each other) which suggested they were mildly polluted. Finally, two small, shallow lowland rivers with high levels of anthropogenic impacts

(grazing, agriculture, urbanised and industrial areas) surrounding them had significantly different invertebrate community structure from all other sites. Certain taxa including Chironomidae (midges), Hirudinae (leeches), Planorbidae (snails), *Physa acuta* (air-breathing freshwater snails), *Cura sp.*(flat worms), Ceinidae (amphipods), Paramelitidae (amphipods) and Oligochaeta (aquatic worms) were indicators for sites rated as *mild to moderate pollution* (lowland rivers) while Scirtidae (beetles), Hydrobiosidae (caddisflies), Leptophlebiidae (mayflies), *Eusthenia costalis* (stoneflies), and Elmidae (beetles) were indicative of cleaner sites (upland rivers). The spatial differences in communities among rivers were mostly due to differences in number of each taxa within community. The temporal comparison showed that there were similarities in invertebrate community between summer and autumn as well as between winter and autumn. The largest temporal differences in communities occurred prior and post a large flood event highlighting the role of natural disturbance in affecting stream macroinvertebrate communities.

Chapter 3 compared the macroinvertebrate communities in autumn 2016 in northern and southern streams with and without farms to examine the impacts of farm effluents at the farm outlet on the receiving stream. Outfalls at the aquaculture sites at Patricks, Brumbys, Russell Falls and Florentine showed similarities in community structure with high numbers of pollution tolerant species (Oligochaeta, Planorbidae, *Physa acuta,* Sphaeriidae (freshwater bivalve molluscs), Hirudinae, and Chironomidae) but significant differences to upstream reference sites as well as other non-aquaculture sites, highlighting the impacts of nutrient waste in aquaculture effluent on receiving waters. Furthermore, outfalls on Brumbys and Florentine appeared to have a greater impact than outfalls on the Patricks and Russell Falls sites. In contrast, the Broad as well as the upstream sites of Patricks, Russell Falls and

Florentine all surrounded by forest had a similar community structure and were the cleanest sites with high numbers of *Eusthenia costalis,* Baetidae and Scirtidae. The community structure of the upstream reference site on the Brumbys was similar to the non-aquaculture Derwent, Dee and Ouse while the Tyenna End and the Styx also showed similar community structure. These six sites indicated mild to moderate pollution, highlighting the presence of other sources of pollution.

Chapter 4 determined the differences in macroinvertebrate communities among stations at different distances downstream from the farm outlet in Brumbys Creek and Florentine River over four seasons to describe the level of impact and degree of recovery. The most impacted stations were at the waste discharge point and station immediately below, which showed significant differences in macroinvertebrate community structure from other stations. Those two stations had high numbers of pollution tolerant species, high numbers of total individuals; but lower taxa richness and lower diversity. Although macroinvertebrate composition of the stations further downstream differed from upstream and outlet stations; the communities overlapped suggesting a recovery in the health of the stream moving downstream. However, this was only observed > 800m from the outlet. In terms of indicators species, Psephenidae (water-penny beetles), Gripopterygidae (stoneflies), *Eusthenia costalis* (stoneflies), Ceinidae, Elmidae, Baetidae, Paramelitidae and Leptoplebiidae were indicative of non-farming conditions (upstream stations); Oligochaeta, Planorbidae, *Physa acuta*, Hirudinae and Ancylidae (air-breathing limpets) were indicators of polluted conditions at the outlet and just below the outlet; while Orthocladiinae (midges), Tanypodinae (midges), Chironominae (midges) and Hydropsychidae were indicative of *mild pollution* in stations further downstream.

Chapter 5 describes the correlation between stream macroinvertebrate communities and physical water parameters. The first objective was to examine whether water chemistry might be a proxy for the macroinvertebrate communities in Tasmania or if specific macroinvertebrates might actually be the best (most cost-effective) tool for a monitoring program. The second objective was to determine potential bioindicators correlated with specific water parameters to use as a simple and quick tool for farms to manage and control aquaculture impacts. There was a relationship between macroinvertebrates and water quality chemistry. The less disturbed sites (upland rivers) had a higher abundance of pollution intolerant taxa (notably Psephenidae, Baetidae, *Eusthenia costalis*, Gripopterygidae, *Atalophlebia australis*, *Costora Delora*, *Lingora sp.* and Scirtidae) which correlated with DO and pH levels greater than 9 mg/l and 7 respectively and indicated good water quality. Aquaculture sites appeared to have markedly higher nitrogen and phosphorus concentrations compared to other sites with ammonia, nitrate, nitrite, nitrate & nitrite, total phosphorus at farming sites ranging from 210 – 580 ug/l, 0.06 – 0.17 mg/l, 0.011 – 0.25 mg/l, 72- 200 ug/l, and 40 – 80 mg/l respectively. These high concentrations were correlated with high abundances of Oligochaeta, Planorbidae, *Physa acuta*, *Cura sp.* and Hirudinae. Tipulidae (crane fly), Ceinidae, Paramelitidae, Caenidae (mayflies), Hydrobiosidae (caddisflies), Ecnomidae (caddisflies), Sciomyzidae (marsh flies), Hydroptilidae (caddisflies), and Calamoceratidae (caddisflies), suggesting these taxa were indicators for agriculture and grazing sites (lowland rivers). Nevertheless, water chemistry showed no marked differences between agriculture sites (alternative sources of impacts) and the clean forest sites; while macroinvertebrates were different between these sites suggesting macroinvertebrates may be better than water chemistry in terms of a monitoring tool.

In summary, the results of this study have increased the understanding in Tasmania of how macroinvertebrates respond to different geomorphology, natural habitat and pollutants and can identify recovery at distances moving downstream from the outfalls. The major flood that occurred in 2016 had a significant influence on macroinvertebrates at both aquaculture and non-aquaculture sites in the southern region, with different macroinvertebrate communities observed after the flood, in contrast to only a slight impact on communities in the northern region. Aquaculture effluents had potential impacts on rivers; however, the level of impact decreased moving downstream from the outfalls. Overall, the recovery level appears to depend on the amount of waste discharge, stream conditions and the distance from impacted points. Therefore, establishing stream baseline standards is important to evaluate both impact and recovery processes. Finally, using indicators species appears to be a quick, simple and cost-effective tool to assist aquaculture farms wishing to assess and monitor effluents for management and regulatory purposes. Instead of looking at the whole community at a site, the presence and the degree abundance of taxa such as Oligochaeta can indicate the degree of pollution.

Acknowledgements

The completion of this PhD was a wonderful and challenging experience. During the 4 years, many people were directly and indirectly involved and supported me to shape my academic career.

First and foremost, I would like to express my gratitude to my supervisors, Catriona Macleod, John Purser and Jeff Wright, for providing their invaluable expertise, guidance and support. I have appreciated the amount of time that all of them have spent guiding me throughout the duration of this Ph.D, especially their valuable advice given during the thesis writing, scientific editing and proof reading; as well as their efforts checking and editing my writing structure and grammar. I would not be here today without their patience and significant contributions. I would particularly like to thank Catriona for giving me her time during her most difficult time while taking care her husband, Henry (Harry) King, during his critical illness. Special thanks to John for helping me in sampling macroinvertebrates at ten sites, even when the weather was rainy and cold. I also want to thank Jeff for his unhesitating decision to be my primary supervisor once John and Catriona could not keep the position.

I would like to express my sincere thanks to my research advisor, Tony Furlonge (NRM North), who played vital role in the macroinvertebrate identification. I very much appreciated her time and effort to check my reference specimens for correct identification as well as her encouragement.

The support of the University of Tasmania must be acknowledged. I would not reach this Ph.D without the given scholarship.

I would like to thank the agencies; Department of Primary Industries, Parks, Water and Environment (DPIPWE), Environment Protection Authority (EPA), the Derwent Estuary Program (DEP) incorporating DEP, Hydro Tas, Natural Resource Management (NRM South) and TasWater; for supporting the project and sharing data. I would also like to acknowledge the individual support of the former scientist of DPIPWE, Jason Whitehead, and thank him for advising on sampling sites as well as helping with the data sharing agreement. Thanks to Christine Coughanowr (DPIPWE), Scott Hardie (DPIPWE), David Horner (EPA/DPIPWE), Sarah Richards (EPA/DPIPWE), and Gregory Dowson (EPA/DPIPWE) for helping me with database access as well as macroinvertebrate identification and Chlorophyll-a sampling. Thanks to Nepelle Crane (NRM South) for inviting us to the River Derwent Plan Key Stakeholder Workshop which provided information for my project within the Derwent River Catchment.

The support of the Tassal Pty Ltd, Petuna Aquaculture Pty Ltd, and Mountain Stream Fishery must be acknowledged. Macroinvertebrate sampling could not be performed without their support and permission. In particular, I would like to recognise the support of Mark Hilder (the former manager based at Saltas, Tassal Group), Dr Ryan Wilkinson (the former manager, Petuna Group) and Ryan Hagen (WHS Co-ordinator, Petuna Group) for providing us with the most favourable conditions during our sampling times. Thanks to Matt Barrenger (Senior Manaer – Environment, Tassal Group) and Jessica Widdison (Environmental Officer, Tassal Group) for helping me with data sharing agreement between Tassal Group and the University of Tasmania.

Several other people have also made notable contribution to this Ph.D. I would like to thank Bernadette Proemse (UTAS) and Anne Watson (UTAS) for guiding and helping me with the Chlorophyll-a sampling technique and laboratory analysis. Thank you so much to Melanie Leef

(UTAS) and Jon Schrepfer (UTAS) for providing good conditions in the laboratory as well as sampling gear for the research. I would like to express my very great appreciation to Martin Filleul for his support during the remaining eight sampling trips while John could not attend. Special thanks to Jason Beard (UTAS) for advising and instructing me with data analyses of chapter 5 (correlation between macroinvertebrate and water quality data). Thanks to Ngoc Tran (Ph.D candidate) for helping me with macroinvertebrate processing which was time-consuming.

Finally, special mention must be made to my family for your support and encouragement; especially my husband, Tan, and my 4-year-old son, Milan, who have understood and unconditionally supported me during my Ph.D.

Explanatory note regarding thesis structure

Chapter 2, 3, 4 and 5 have not been prepared for publication manuscripts at the time of submission. Hence a statement of co-authorship has not been made. The same sampling materials and methods were used in the four research chapters. For brevity, these were stated in detail in chapter 2 but then referred to in Chapters 3-5.

Information on the hatcheries is provided in the appendix (page 278) to gauge the intensity.

Taxonomic level of study depends on available keys as some Australian species have not been studied very well (ie keys are poorly developed for some taxa); which was also explained on the Methodology method (Chapter 2, page 30).

The references for each chapter can be found in the cumulated bibliography starting on page 250.

Table of Content

List of tables

Table of Figures

1 Chapter 1: General introduction

1.1 Aquaculture status

Global aquaculture has expanded rapidly over the previous few decades to become highly intensive (Bostock et al., 2010). From 1980 to 2010, there was a twelve fold expansion in aquaculture production, with an average growth rate of 8.8% per year (FAO, 2018). Global aquaculture accounted for only 30% of the world's fish food supply in 2000 (Boyd, 2003b), but this has steadily increased over the last few years; to 44% in 2008, 45% in 2009, 47% in 2010 and 49% in 2011 (FAO, 2018). In 2008, worldwide production of aquaculture totalled 52.5 million tonnes (Bostock et al., 2010) and by 2009 this figure increased to 55.7 million tonnes before continuing to rise to 80 million tonnes in 2016 (FAO, 2018). However, the rapid development of aquaculture has brought about a growing concern regarding potential environmental impacts caused by a significant increase in the use of a large quantity of fish feed with resultant waste products (Aure and Stigebrandt, 1990; Bostock et al., 2010; Gowen and Bradbury, 1987; Kelly et al., 1996; Tacon and Forster, 2003).

1.2 Environmental issues

Aquaculture can have negative effects on the surrounding environment (Bostock et al., 2010; Naylor et al., 2000). This is due to nutrient and solid waste discharge from fish farms (Aure and Stigebrandt, 1990; Bostock et al., 2010; Gowen and Bradbury, 1987; Kelly et al., 1996) generated by uneaten feeds, fish excretion, faecal material, soluble metabolites, medications and pesticides (Bergheim and Selmer-Olsen, 1978; Carroll et al., 2003a; Kelly et al., 1996; Kendra, 1991; Wu, 1995). Intensive fish farming can cause adverse effects on water quality, seabed enrichment (Wu et al., 1994), and a reduction in ecosystem health and biodiversity (Bostock et al., 2010). Furthermore, aquaculture activities can pollute fish farms themselves,

nearby farms or entire waterways (Silvert, 1992). Larger scale effects can be due to oxygen depletion and benthic deposition of faecal matter and unconsumed food pellets at numerous closely located cages causing larger-scale eutrophication and toxic algal blooms influencing entire water bodies in the area (Bonsdorff et al., 1997).

The volume of feed usage has increased considerably with a growing fish production as a result of the growth of aquaculture (Bostock et al., 2010). Consequently, both marine and freshwater fish farming can have the potential to affect the environment. While the majority of fish produced worldwide are cultured in freshwater ponds, however little has been reported in the literature on their impact on the environment. Most environmental studies have focused on marine cage aquaculture mainly due to the rapid expansion of salmon farming. The following sections will outline the impacts of aquaculture and some of the mitigation strategies to reduce or minimise impacts.

1.2.1 The impact of marine cage farming on the environment

A large amount of faecal excretion, uneaten feed and organic detritus generated by cage farming is deposited on the seabed leading to organic enrichment and nutrient loadings, potentially overwhelming the natural feeding capacity of benthic animals (Buschmann et al., 1996; Carroll et al., 2003a; Gowen and Bradbury, 1987; Gowen et al., 1990; Holmer and Kristensen, 1992; Karakassis et al., 2000; Silvert, 1992; Wu, 1995). Excessive feed input may induce eutrophication leading to anoxic conditions, which may allow for the proliferation of bacterial mats and lead to hydrogen sulphide and methane production (Brown et al., 1987; Hall et al., 1992), the enhancement of sediment metabolism, high ammonium efflux (Hargrave, 1993; Holmer and Kristensen, 1992), and may be associated with algal blooms, including the potential for harmful algae (Wu, 1995). As a consequence, cage farming can

cause water quality degradation (high nutrient levels, turbidity, and organic matter, and reduction in pH level) (Brown et al., 1987; Hall et al., 1992), benthic and macrobenthic fauna depletion or impoverishment (changes in biomass, abundance and diversity) under cages and nearby areas (Brown et al., 1987; Pillay, 2008; Silvert, 1992; Ye et al., 1991).

1.2.2 The impact of freshwater fish farming on the environment

Land-based effluents also have potential environmental impacts on receiving waters, including both positive and negative effects. Nutrient and solid waste discharge can be food for floral and faunal communities in receiving waters resulting in an increase in the number and composition of macroinvertebrates that can in turn be food for higher animals such as fish (Bennison et al., 1989; Nobre et al., 2010). However, excessive waste products from land-based freshwater farming can cause pollution in streams, rivers, lakes, estuaries and coastal areas (Koçer et al., 2013; Pillay, 2008) as the nitrogen, carbon and phosphorus components in feed exceed fish requirements during digestion (Ackefors and Enell, 1994; Gowen and Bradbury, 1987). Evidence has shown that, waste products from fish metabolism and unconsumed feeds (Kendra, 1991; Rerat and Kaushik, 1995), and chemical residues from therapies (Capone et al., 1996; Kerry et al., 1995; Smith et al., 1994) can deteriorate the water quality downstream of trout farms (Cornel and Whoriskey, 1993; Pillay, 2008; Selong and Helfrich, 1998). This may lead to a reduction in dissolved oxygen (DO) (Bergheim and Sivertsen, 1981; Enell, 1987; Rennert, 1994; Selong and Helfrich, 1998) and pH (Bergheim and Sivertsen, 1981; Brown et al., 1987; Hall et al., 1992; Rennert, 1994), and a rise in biological oxygen demand (BOD), ammonia (NH_3), and nutrients (Bergheim and Sivertsen, 1981; Enell, 1987; Pillay, 2008; Pulatsu et al., 2004a; Rennert, 1994; Selong and Helfrich, 1998). Thus, highly polluted water quality typically occurs at the farm outlet and decreases as one moves

downstream (Camargo, 1993; Camargo, 1994). Furthermore, effluents can reduce the population and composition of the bacteriological flora (Husevåg et al., 1991), phytoplankton, zooplankton and benthic invertebrates (Cornel and Whoriskey, 1993; Pillay, 2008). At some polluted sites there has been a reduction in the species richness of macroinvertebrates and decreased abundance of sensitive 'clean water species' (Pillay, 2008), and an associated increase in pollution-tolerant non-insect taxa downstream of farms (Selong and Helfrich, 1998). The number of macroinvertebrate families is lower at, and downstream of, discharge points compared to upstream, but the total number of individuals are typically highest at the farm effluent and lowest at upstream stations (Camargo, 1993). From the farm outlet and areas immediately downstream, the abundance of certain families such as Chironomidae (midge larvae), Planorbidae (snail), Sphaeriidae (pea shells), Baetidae (mayflies), Tipulidae (tipulids), Empididae (flies) and Simuliidae (black flies) increased significantly (Camargo, 1993) and are tolerant of pollution (Chessman, 2003a; MDFRC, 2009). In comparison, the abundance of Elmidae (riffle beetles) and Leptophlebiidae (mayfies) decreased substantially from upstream to downstream or were absent at downstream stations (Camargo, 1993) and appear sensitive to pollution (Chessman, 2003a; MDFRC, 2009). Although initial enrichment of the environment may increase the habitat diversity, further build-up of wastes (especially solid waste) will reduce benthic animal composition and deteriorate water quality.

1.2.3 Mitigation strategies

Mitigation strategies have been applied to minimise the negative impacts of effluents from land-based farms on receiving waters. Settlement ponds can settle solid wastes, but do not address soluble waste (Jegatheesan et al., 2007; Tacon and Forster, 2003). Recirculating

Aquaculture Systems (RAS) can reuse water and remove waste through mechanical filtration and biofiltration (Ebeling and Timmons, 2012; Nazar et al., 2013). Another popular way to remove wastes is by utilising micro-screen filters to remove suspended solids (Ebeling et al., 2004). Integrated cultivation, using more than one species of aquatic animals or both aquatic animals and plants (aquaponics), has also been applied (Neori et al., 2007; Troell et al., 1997; Wu, 1995). Macrophytes, bivalves, molluscs and herbivorous fish (seabream, carp, tilapia) have the capacity to harvest nutrients and pollutants in effluents and thereby, improve the water quality in finfish farms. Not only can the integrated cultivation reduce such nutrients and pollutants, it can also increase the profitability for farmers through the yield of those associated species. Reduction in the utilisation of high-risk feed items such as trash fish and invertebrates, and chemicals such as antibiotics, herbicides and pesticides are also considered as mitigation approaches (Tacon and Forster, 2003). Improved artificial feed formulation, using higher energy fish feeds to reduce the tonnage of feed used (Heinen et al., 1996; Ingram, 1999), improved feed distribution and feeding strategies have also been recommended to reduce environmental impacts (Ingram, 1999; Wu, 1995). However, mitigation strategies may not address all solid waste of farms, thus monitoring tools play an important role to evaluate and inform approaches to manage impacts of effluents on receiving water.

1.3 Function of macroinvertebrates in environmental assessment

Although fish farms may have applied mitigation approaches, it is important to evaluate the effectiveness of these farm management practices. Biological monitoring approaches using bacteria (Boaventura et al., 1997; Carr and Goulder, 1990a; Schmidt et al., 2000), microalgae (Ashton and Richardson, 1995), macrophytes (Camargo et al., 2011; Carr and Goulder, 1990b; Daya and Pant, 2017), periphyton (Coste et al., 2009; Li et al., 2010), water quality (Azrina et

al., 2006; Camargo, 1993; Camargo, 1994; Fries and Bowles, 2002; Hardie et al., 2012; Pulatsu et al., 2004b), fish (Fierro et al., 2017; Houle et al., 2012; Roset et al., 2007; Schiemer, 2000) and macroinvertebrates (Balderas et al., 2016; Barnes et al., 2013; Camargo, 1993; Chará-Serna et al., 2015; Daya and Pant, 2017; Fierro et al., 2017; Humphries, 1996; Humphries et al., 1996; Kırkağaç et al., 2009; Metcalfe, 1989; Nobre et al., 2010; Romero et al., 2017; Roy et al., 2003b; Slooff, 1983; Spruzen et al., 2008; Storey et al., 1991) have all been used to assess the effects of environmental stressors on stream health as well as the impacts of fish farms on receiving waters. Moreover, the tendency of establishing such a national DNA barcode database for macroinvertebrates in freshwater bioassessment has been considered for bio-surveillance in the world (Elbrecht et al., 2017) and in Australia (Carew et al., 2017; Shackleton and Rees, 2016). This can provide accurate species identification as DNA barcodes are short, standard amplified fragments (Carew et al., 2017), however, the method currently lacks laboratory protocols and reference databases.

Within this suite of biological surveillance approaches, the most common methods used to determine the potential for negative environmental impacts are either assessment of water quality parameters (DO, pH, BOD, NH_4-N, total phosphorus (TP), total nitrogen (TN)) or living organism monitoring.

Algae are also another reliable tool and have been used to assess environmental impacts in aquatic habitats for a long time throughout the world (Stevenson and Smol, 2003). For instance, phytoperiphyton was used to assess water quality in north–western Russian rivers (Komulaynen, 2002) and in South Africa (Harding et al., 2005), to assess trophic status in Irish lakes (DeNicola et al., 2004); to examine nutrient and organic enrichment in flowing waters (Porter et al., 2008); to indicate stream total N and total P concentration (Winter and Duthie,

2000), pollution in the lower Jordan River, Israel (Barinova et al., 2010); and to survey the influence of urban and agriculturally stressed rivers on diatom community structures (Walsh and Wepener, 2009). Furthermore, macroinvertebrates and algae have been used in combination as rapid bioassessment tools (Barbour et al., 1999; Carew et al., 2017; Chessman et al., 1999; Prince, 1995). Firstly, they have a short lifespan with an average life expectancy ranging from a few days to a year, and so the assemblages and distribution of benthic algae will be different in response to variations in water quality (OME, 2012). Fetscher et al.(2009) stated that algae can be used as a rapid assessment tool and indicators for stream recovery because they could respond quickly to changes in the environment. Secondly, they can also be used to diagnose the causes of environmental impairment such as heavy-metal contamination, organic enrichment, or siltation (Fetscher and McLaughlin, 2008). With high dispersal rates, growth rates, and relatively short generation times algae respond rapidly to environmental changes. However, there have been some disadvantages to using algae as a sensitive indicator, including severe effects being limited to 50 m from the outfall, sampling being extremely expensive to conduct (specialized equipment in the field and specialized laboratory) (Carroll et al., 2003b), and algae being difficult to quantify and taxonomically challenging (Fetscher et al., 2009).

There are some operational factors that also need to be considered when selecting a monitoring approach, for example water quality will fluctuate in response to daily husbandry activities such as feeding and cleaning (Kaushik and Cowey, 1991) and may be most appropriate if short-term fluctuations are important. In contrast, macroinvertebrates may better show the long-term effects of any environmental changes (Goodnight, 1973). Moreover, most macroinvertebrates (as opposed to micro-invertebrates) are larger than 500

microns and therefore are visible to the naked eye (De Pauw et al., 2006) making them convenient for field inspection, storage and transfer (Chessman, 1995). Therefore, biological monitoring using macroinvertebrates (Balderas et al., 2016; Bennison et al., 1989; Chessman, 1995) has been widely used to indicate the quality of water (Smith et al., 1999) as well as the health of aquatic environments (Bennison et al., 1989; Slooff, 1983). This is because their assemblages reflect the long-term water quality parameters, with compositional change associated with degrees of water quality and hence they can act as indicator species (Bennison et al., 1989; Slooff, 1983).

Additionally, different macroinvertebrate taxa have high sensitivities and low tolerances to organic enrichment, chemicals, water quality and pollutants, and hence will be positively or negatively affected by these changes (Azrina et al., 2006; Camargo, 1994; Chessman, 1995; Goodnight, 1973; Metcalfe, 1989; Slooff, 1983). Different taxa of macroinvertebrates consume different types of food such as organic and inorganic matter, algae, and aquatic plants. Therefore, changes in the macroinvertebrate community composition adequately reflect the impacts of feeds, organic matter, chemicals and various pollutants on the aquatic environment. They are generally indicative of specific environmental quality, even if there is a low abundance of macroinvertebrates or absence of pollutant factors at the sampling time (Azrina et al., 2006; Bennison et al., 1989; Chessman, 1995; Metcalfe, 1989). Life cycles of macroinvertebrates range from a few weeks to a few years, and their larval stages are comparatively sedentary making them suitable monitoring species as they cannot change community composition or be quickly absent when pollution factors change or disappear. As a consequence, the presence of specific macroinvertebrates can be representative of local conditions (Chessman, 1995; Goodnight, 1973; Marchant, 1986; Metcalfe, 1989). For

instance, aquatic worms (e.g. class Oligochaeta, family Planariidae, genera *Tubifex* and *Limnodrilus*), family Tubificidae, freshwater leeches (class Hirudinea), and larvae and pupae of midges (Chironomidae) are strong indicators of organic pollution (Chessman, 2003a; Gooderham and Tsyrlin, 2002; Goodnight, 1973). The ratio of *Gammarus* (Amphipoda) : *Asellus* (Isopoda) present in a stream can be indicative of organic pollution (Whitehurst, 1991) whereas the presence of mayfly larvae (*Baetis rhodani* and *B.vernus*) can be representative of heavy metal pollution in streams (Fialkowski et al., 2003). Many taxa of snails (e.g. *Physa spp.*) are generally present in septic streams while freshwater bivalve molluscs (Sphaeriidae) are indicators of low oxygen conditions. In contrast, mussels, freshwater clams (family Corbiculidae), mayflies (family Leptophlebiidae), stonefies (family Notonemouridae) and caddisfies (families Hydrobiosidae, Odontoceridae and Hydrosychidae) are generally found where water quality is good (Chessman, 2003a; Gooderham and Tsyrlin, 2002; Goodnight, 1973).

Because of these predominant features, biological monitoring using macroinvertebrates has been applied globally, to assess both marine and freshwater environments. Macroinvertebrates have been primarily used to assess water quality (Borja et al., 2009; Camargo, 1993; Camargo, 1994; Cao et al., 1996; Metcalfe, 1989) or as an indicative and valuable planning tool to manage water uses, monitor ambient environment and evaluate the effectiveness of pollution control measures (Chessman and McEvoy, 1997; Metcalfe, 1989). Macroinvertebrates are observed and analysed for ecological assessment of rivers (Buffagni et al., 2000; Clarke et al., 2003; Palmer et al., 1996; Smith et al., 1999), streams (Conor Keitzer and Goforth, 2013; Goodnight, 1973), and lakes (Moore, 1979). They have also been used to link effects on the environment of effluents from land-based marine fish farms (Silva et al.,

2013), freshwater farms (Kırkağaç et al., 2009), and hatcheries (Fries and Bowles, 2002). Macroinvertebrates have been used as a standard tool in the Biological Monitoring Water Quality (BMWQ) score (Armitage et al., 1983b; Camargo, 1993), and are currently used in the Australian River Assessment System (AUSRIVAS) monitoring as part of the Australian River Health Monitoring program (Parsons et al., 2002).

Different sampling devices also play an important role in biomonitoring programs, their selection determined by the ability to gain a representative sample and their applicability to habitat to be sampled. There have been many sampling techniques applied to sampling stream macroinvertebrates (Brua et al., 2011), including Hess cylindrical sampler (Camargo, 1994; Sponseller et al., 2001b), Van Veen grab (Edgar et al., 2005; Silva et al., 2013), D-framed pond net (Fries and Bowles, 2002; Smith et al., 1999), corer (Hirst et al., 2006; Winberg et al., 2007), kick sampler (Fries and Bowles, 2002) and Surber sampler (Hardie et al., 2012; Smith et al., 2009). Two of the most common techniques used to collect macroinvertebrates are the Surber sampler (Hardie et al., 2012; Humphries et al., 1996; Roy et al., 2003b; Smith et al., 2009) and kick-sampling (Barnes et al., 2013; Fries and Bowles, 2002). These two sampling techniques have also been applied to the AUSRIVAS monitoring program. A Surber sampler is a quantitative sampling method whereas the kick-sample method using a standard sample net is considered semi-quantitative (Barbour et al., 1999). Diversity, biotic indices, multi-metric approaches, multivariate approaches, functional feeding groups (FFGs) and multiple biological traits are most frequently used approaches to determine stream and river health (Li et al., 2010; Oliveira and Cortes, 2006).

1.4 Tasmanian salmonid aquaculture

Australian aquaculture production has gradually increased, rising from 54,652 tonnes in 2005 – 2006 to 97,046 tonnes in 2015 – 2016 (FRDC, 2017). Salmonids, prawns, oysters, southern bluefin tuna, barramundi and yellow tail kingfish are commercially farmed species that have contributed substantially to Australian aquaculture production (FRDC, 2017; Walker, 2014). In 2015-2016, the Australian salmonid industry produced 53,319 tonnes of Atlantic salmon which was more than twice higher than in 2005 – 2006; making up 78% of the gross value of aquaculture production, and was the highest volume in Australian aquaculture (FRDC, 2017).

Tasmanian aquaculture contributes the largest volume by state and constitutes 98% of the Australian salmonid production in 2015 – 2016 (FRDC, 2017). Salmon farming was initially introduced into Tasmania in the mid-1980s and has grown significantly since then. The first harvest of farmed commercial salmon was 53 tonnes in the summer of 1986-1987 (TSGA, 2019) and is currently around 54,772 tonnes accounting for $704 million in 2015 - 2016 (FRDC, 2017). Commercial production of rainbow trout in freshwater farms began in the 1964 (Inland Fisheries Service) and in cages in marine/estuarine locations as 'Ocean' trout in the 1980s. Farmed salmonids, including Atlantic salmon and rainbow trout have since become the largest aquaculture sector in Tasmania. The salmonid industry has provided the State with overwhelming economic advantages and has made a significant contribution as a quality producer of fine foods, adding to Tasmania's reputation. This industry currently provides more than 2,292 direct jobs and 6,270 indirect jobs; accounting for $190 million of the Tasmanian Gross State Product (TSGA, 2019). The Tasmanian salmonid industry currently produces around 63,000 tonnes per annum worth $810 million at wholesale levels (TSGA,

2019) with over 11 million smolts and fingerlings produced in 16 freshwater hatcheries (land based fish farms) to supply sea-cage production in Tasmania (Walker, 2014).

1.5 Tasmanian jurisdictions

The Tasmanian aquaculture industry operates under the jurisdiction of Inland Fisheries Service (IFS); Department of Primary Industries, Parks, Water and Environment (DPIPWE); Parks and Wildlife Service (PWS), and the Tasmanian Environmental Protection Agency (EPA). Under the Inland Fisheries Act 1995, the IFS mission is to manage and develop inland fishery resources in Tasmania; and retain jurisdiction over freshwater fish in all inland waters. The IFS is responsible for regulating and promoting commercial freshwater fisheries and aquaculture ventures, approving the movement of fish throughout the state, approving the importation of exotic fish into the state (e.g. for research or aquarium fish), managing pest fish and protecting native freshwater fauna (IFS, 2013).

DPIPWE has played an important role in assessing and monitoring water quality in rivers, including pesticide and water monitoring, and hydrological and water assessment. Their monitoring program ensures the sustainability of agricultural practices and assists management decisions about water allocation, river conditions, water quality and aquatic ecosystems (DPIPWE, 2014). The Department of the Environment protects and manages important flora, fauna, ecological communities and places of heritage under the Environment Protection and Biodiversity Conservation Act 1999 (DE, 1999).

The Environment Protection Authority (EPA) is Tasmania's principal environmental regulator. The EPA's aim is to regulate developments and activities that may impact on environmental quality and to promote best practice, sustainable environmental management. The EPA

administers the Environmental Management and Pollution Control Act 1994 and is an integral part of Tasmania's Resource Management and Planning System; which inform environmental assessment decisions, through an efficient integrated assessment process and to regulate to ensure that major industrial, municipal and community activities employ best practice environmental management. In particular EPA has been having a remit to monitor the intended expansion and development plans of the salmon industry.

The Australian River Assessment System (AUSRIVAS) is responsible for assessing the biological health of Australian rivers under the National River Health Program (NRHP), a Federal Government program. In particular, Stream Invertebrate Grade Number—Average Level (SIGNAL) has been used to indicate the health status in Australian rivers since 1993. SIGNAL is a biotic index using different macroinvertebrate taxa reflecting differences in pollution tolerance levels to score the water quality in rivers (Chessman, 1995; Chessman, 2003a; Chessman, 2003b). The original SIGNAL was easily applied in south-eastern Australia, but was difficult to use for northern, western and inland Australia. Therefore, SIGNAL 2 was developed by the National River Health Program to be applied to all Australian rivers and it has been subsequently adopted for use for the monitoring programs of AUSRIVAS and the Murray-Darling Freshwater Research Centre (MDFRC). The SIGNAL 2 score is calculated based on total number and types of macroinvertebrate taxa. Each macroinvertebrate taxon is given a number from 1 to 10 depending on its pollution intolerance or tolerance, which was considered as a SIGNAL 2 grade. Pollution tolerant families have low SIGNAL 2 grades while families sensitive to pollution have a higher SIGNAL 2 grade (Chessman, 2003a). SIGNAL 2 grades were set for approximately 171 families and 33 higher taxa (Chessman, 2003b). High

scores of SIGNAL 2 generally represent systems with better water quality such as low salinity, turbidity and nutrient levels (Chessman, 2003a).

While Tasmanian salmon producers have applied different waste management methods such as RAS, settlement ponds, filtration devices and waste water treatment systems to reduce the effects of waste product discharge on receiving water, companies are still required to comply with environmental standards set by government agencies. In light of flagged industry expansion plans, it is therefore beneficial to develop monitoring programs which will assist the management of farm outputs and receiving waters complementing the current in-house monitoring undertaken by salmon companies to comply with EPA requirements. Factors involved in minimum requirements from EPA include biological monitoring parameters (macroinvertebrates, stream shading, algal cover and chlorophyll a); and physical and chemical water quality parameters (dissolved oxygen mg/L, dissolved oxygen % saturation, field conductivity @ TRef25 µS/cm, pH field - sensor TC, turbidity NTU, temperature (celsius), ammonia as N mg/L, nitrate as N mg/L, nitrite as N mg/L, total nitrogen as N mg/L, phosphorus dissolved reactive as P mg/L, total phosphorus mg/L, and total suspended solids).

1.6 Thesis aims

This thesis had four main aims:

1. To determine whether stream macroinvertebrate communities differed among non-farm sites in two regions of Tasmania (north and south) and across four times.
2. To compare the macroinvertebrate communities at one time (autumn 2016) in northern and southern streams with and without farms in order to examine the impacts of farm effluents on receiving streams.

3. To determine the differences in macroinvertebrate communities among stations at different distances downstream from the farm outlet over four seasons in two rivers with farms (Brumbys Creek and Florentine River) to describe the level of impact and degree of recovery.
4. To describe the correlation between stream macroinvertebrate communities and physical water parameters in the Derwent River Catchment to examine whether water chemistry might be a proxy for the macroinvertebrate communities and to determine potential bioindicators correlated with specific water parameters. Ultimately, this aimed to determine whether specific macroinvertebrates might be used as a simple and quick monitoring tool for farms to manage and control aquaculture impacts.

2 Chapter 2: Spatial and temporal patterns of macroinvertebrate communities

Abstract

Macroinvertebrates were sampled at ten non-aquaculture sites of North (Brumbys Creek and St Patricks River) and South (Florentine, Tyenna at Russell Falls, Broad, Tyenna End, Styx, Dee, Ouse, Derwent Rivers) Tasmania to determine the differences or similarities in macroinvertebrate assemblages between rivers in the North and South of Tasmania. Triplicate one-minute kick samples were taken either seasonally (North) or in summer and autumn in 2016 and 2017 (South) at each site, sorted, identified and counted. Multivariate analysis, SIGNAL 2 index, biological variables (relative abundance, total abundance, taxa richness and Simpson diversity index) were employed to analyse the data. Macroinvertebrate community composition showed a high degree of similarity between the two regions (North and South) with the two sites in the north being more similar to sites in the south than to each other. Differences in macroinvertebrate communities between sites presumably reflected differences in stream geomorphology, natural habitat, and biological conditions. The St Patricks (North), Broad (South), Tyenna at Russell Falls (South) and the Florentine (South) which are all upland streams with a sandy and rocky substrate and surrounded by forests, had similarities in macroinvertebrate communities and were indicative of better water quality ratings. The Brumbys (North), Tyenna End (South), Styx (South) and the Derwent (South) are located in lowland grazing and agricultural areas, were also similar in community composition, and were highlighted as mildly polluted sites. In contrast, the Dee and the Ouse in the South which are small lowland streams with very low water depth, and rocky substrate with reasonable detrital loads in grazing, agricultural, urban and industrial areas, and high

anthropogenic impacts, indicated moderate pollution. Both these sites showed significant differences in invertebrate community from other sites. In the south, there was a large flood event after the 2016 sampling and there were differences in macroinvertebrate community composition between the pre- and post-flood samples at all sites, except the Styx. This study has shown that multivariate analyses such as PERMANOVA and PCO combined with the SIGNAL 2 index were effective for determining differences in macroinvertebrate communities and moreover, they identified indicator species for different water quality ratings between different sites. Based on the comparison of results from all analyses, indicators and SIGNAL 2 scores without a weighting factor appears to be a robust method for the rapid assessment of water quality as it identified water quality ratings based on the types of macroinvertebrates present without relying on counts of abundance.

2.1 Introduction

Freshwater ecosystems are important environments and provide a range of ecosystem services including the provision of water for human consumption and non-consumptive uses as well as providing habitat for aquatic organisms used for food (Postel and Carpenter, 1997). However, many human activities can pollute freshwater ecosystems and threaten these important environments (Dudgeon et al., 2006; Jarvis and Younger, 2000), leading to increasing concern about potential environmental impacts on our freshwater resources. Macroinvertebrates have been widely used as a biological monitoring tool (Bennison et al., 1989; Chessman, 1995) and as macroinvertebrate assemblages reflect the long-term water quality parameters, they can act as indicator species (Bennison et al., 1989; Slooff, 1983). This study uses macroinvertebrates as an assessment approach to characterise freshwater rivers in Tasmania.

2.1.1 The impact of catchment on the environment

Besides aquaculture impacts on the receiving environment (Chapter 1), many studies have shown that human land-use patterns also have the potential to impact stream water quality (Quinn et al., 1997; Sponseller et al., 2001a), habitat, periphyton, and benthic invertebrates (Quinn et al., 1997). Agriculture, urbanization, forestry and climate change have all been shown to impact water quality and present a risk of eutrophication (Hall et al., 1999; Smal et al., 2005) due to an increase of N-deposition and diffusion of non-point source N-inputs to waters (Cirmo and McDonnell, 1997). Rask et al. (1998) found that forestry activities in catchments may increase inorganic and organic loads, the growth of plankton, and abundance of zoobenthos in water; but did not markedly change fish habitat. Moreover, increasing nitrogen and phosphorus efflux in the drainage water because of land clearing and development (Gilliam and Skaggs, 1986) might consequently result in nitrogen saturation (Aber et al., 1989; Aber et al., 1991), water acidification and possible N-based downstream eutrophication (Wright, 1991). The overuse of fertilizer nutrients (organic and mineral nitrogen) (Allan et al., 1997; Beaujouan et al., 2001; Zalidis et al., 2002), pesticides, irrigation, and herbicides from agricultural practices (sugarcane production, cattle grazing and forestry) (Allan et al., 1997; Bramley and Roth, 2002; Zalidis et al., 2002) and drainage water (Evans et al., 1995) has resulted in stream degradation and pollution (Allan et al., 1997; Beaujouan et al., 2001; Bramley and Roth, 2002; Evans et al., 1995) as well as riparian, stream channel habitat and flow alteration (Allan et al., 1997). Although, agriculture may also beneficially enrich receiving water with nutrients and sediments (Tong and Chen, 2002). Often, agricultural (Allan et al., 1997), industrial or urban land use (Allan et al., 1997; Weijters et al., 2009) can negatively influence freshwater biodiversity, leading to a decrease in the number of native freshwater fish and macroinvertebrate species. By way of example, the loss of every

10% of natural land in the catchment resulted in a 6% decrease in species of freshwater fish and macroinvertebrate community (Weijters et al., 2009). Furthermore, changes in invertebrate taxa richness, other biotic indices (Lenat and Crawford, 1994a) and stream macroinvertebrate community structures (Richards et al., 1993) have been shown to be as a result of agricultural practices in the catchment. In addition to biological indices, lotic physico-chemical characteristics such as water flow, suspended sediment, total nitrogen, total phosphorus, nitrate and ammonia outputs can all influence the receiving water (Van Griensven et al. (2006)). The characteristics of the river catchment can therefore both influence the water quality of the river water used for aquaculture and determine the background biota influenced by the farm outfall.

2.1.2 Characteristic of streams examined in Tasmania

2.1.2.1 Northern Tasmania

The St Patricks River is the largest tributary of the North Esk River. It is a small upland stream flowing through State Forest and consists of sandy and rocky substrate. In contrast, Brumby's Creek is a small lowland river, which receives water from the Great Lake on the central plateau via the hydroelectricity scheme at Poatina and flows through agricultural land before joining the Macquarie River. Three weirs are located on Brumby's Creek above the farm site, slowing the water flow. In places where I sampled, water plants such as aquatic milfoil and pondweed grow over a silt or mud bottom overlaying rocks and pebbles.

2.1.2.2 Southern Tasmania (the Derwent catchment)

According to the Derwent Catchment Review (2011), the greater Derwent River catchment is one of the largest river basins in Tasmania and covers approximately 8900 km^2 of south-eastern and central areas of the state (Figure 2.1). In addition, the Derwent River provides

60% of the drinking water supply for Hobart. There are five main sub-catchments in the Derwent catchment: Upper Derwent, Ouse, Clyde, Lower Derwent and Jordan (Figure 2.1). Water is used for hydro-electricity, aquaculture, irrigation, town water, commercial applications, stock usage and for domestic water supplies and recreation. Land use in the Derwent catchment is summarised in Table 2.1.

Table 2.1: Summary of land use in Derwent catchment (Eriksen et al., 2011)

Catchment	Area (km^2)	Conservation % (area km^2)	Forestry incl. Plantations % (area km^2)	Grazing & agriculture % (area km^2)	Other: Urban, mining, industrial % (area km^2)
Upper Derwent	3,561	49% (1745)	35 (1,246)	10% (356)	6% (214)
Lower Derwent	1,517	23% (349)	36% (546)	31% (470)	10% (152)
Ouse	1,478	35% (517)	14% (207)	23% (340)	30% (443)
Clyde	1,131	4% (45)	19% (215)	68% (769)	9% (102)
Total (% of total catchment, km^2)	7687	35% (2,656)	29% (2,214)	25% (1,935)	12% (911)

Figure 2.1: Greater Derwent catchments (Eriksen et al., 2011) excluding the Jordan.

The research objectives of this chapter were to:

1) investigate the differences or similarities in macroinvertebrate assemblages between the targeted rivers in the North and South regions of Tasmania;

2) describe how those communities change over time to suggest the best time for sampling;

3) examine whether macroinvertebrate assemblages in the South were significantly impacted by a major (one in 100 year) flood event and how might this affect monitoring strategies.

2.2 Materials and Methods

2.2.1 Site selection

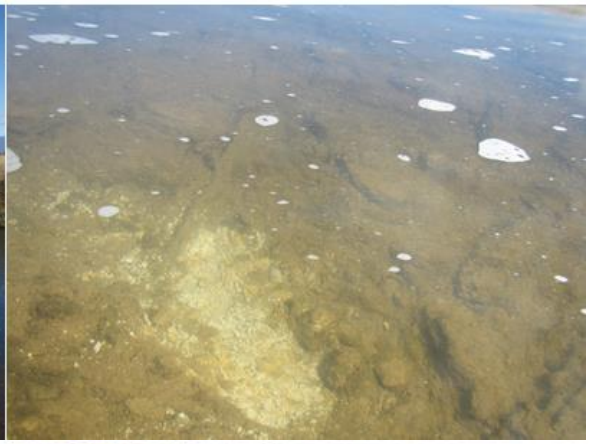
Macroinvertebrate sampling was conducted at a range of nominally unimpacted sites in both North and South Tasmania. Nine streams were sampled: Florentine, Tyenna at Russell Falls, Broad, Tyenna End, Styx, Dee, Ouse, Derwent River (Derwent catchment in Southern Tasmania) and Brumby's Creek and St Patricks River (Northern Tasmania, Figure 2.2). In each river system, only one site was sampled.

Figure 2.2: The map of sampling sites in Tasmania (Google map, 2019).

(1: St Patricks, 2: Brumbys, 3: Florentine, 4: Broad, 5: Dee, 6: Ouse, 7: Russell Falls, 8: Tyenna End, 9: Styx, 10: Derwent)


St Patricks


Brumbys




Broad


Russell Falls


Florentine


Tyenna End


Styx


Dee


Figure 2.3: Natural habitat (left photos) and river bed material (right photos) at ten sampled rivers in Tasmania

Due to a major (one in 100 year) flood event in Tasmania in winter 2016, sampling times differed between the North and the South. Specifically, sampling was undertaken four seasons (summer, autumn, winter and spring) in one year (2016) in the North; and two seasons of summer and autumn in two years (2016 and 2017) in the South as flood made rivers inaccessible. The comparisons of macroinvertebrate assemblages between northern and southern rivers were only carried out in summer and autumn 2016. Experiments then examined season changes in macroinvertebrates in the northern rivers and the impacts of the flood on macroinvertebrates in the southern rivers.

Table 2.2: Description of river bed material of 10 sites in Tasmania based on the classification of Wentworth (1922)

	Rivers	River bed material
1	St Patricks	Pebble with gravel
2	Brumbys	Largely mud under cobbles
3	Broad	Cobble and boulder
4	Russell Falls	Cobble and gravel
5	Florentine	Boulder and cobble
6	Tyenna End	Cobble and pebble
7	Styx	Pebble and gravel
8	Dee	Boulder, cobbles and mud
9	Ouse	Cobble, pebbles and mud
10	Derwent	Bedrock, boulder, cobble and pebble

Northern Tasmania

At Brumby's Creek and St Patricks River (Figure 2.4) macroinvertebrates were sampled at four times in 2016: mid-summer (February 2016), mid-autumn (April 2016), mid-winter (July 2016) and mid-spring (October 2016). Because the flood did not significantly affect northern rivers, the approach focused on seasonal changes.

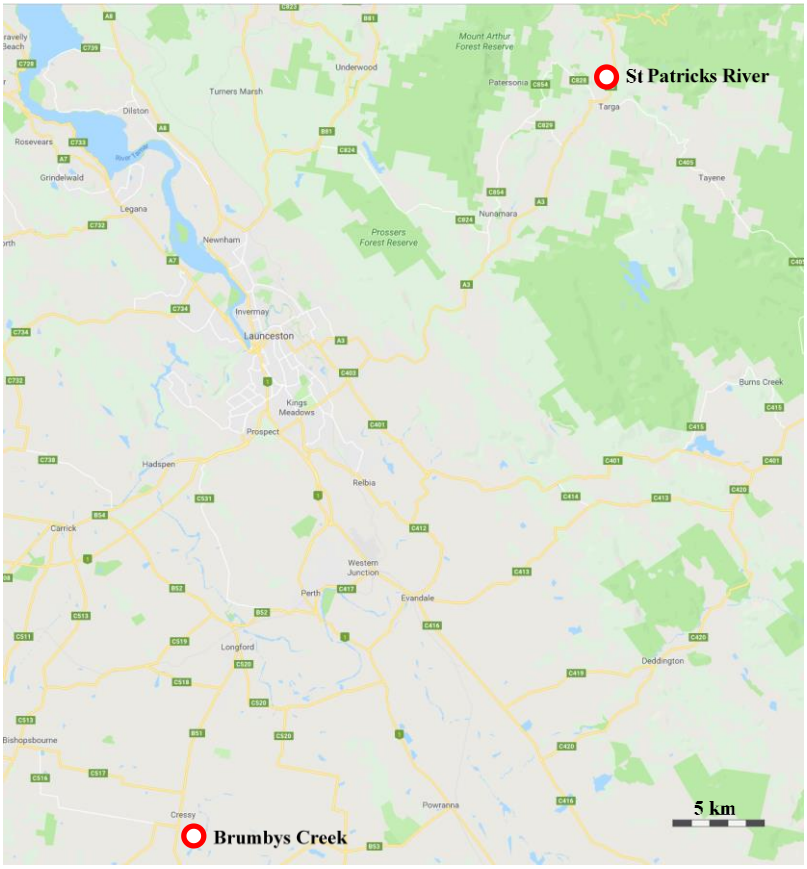


Figure 2.4: The map of two sampling sites in Northern Tasmania (Google map, 2019)

Southern Tasmania

The eight sites in the south (Florentine, Tyenna at Russel Falls, Broad, Tyenna End, Styx, Dee, Ouse and Derwent River, Figure 2.5) were sampled in summer 2016 (February 2016), autumn 2016 (April 2016), and two seasons after the flood: summer 2017 (January 2017) and autumn 2017 (March 2017).

Figure 2.5: The map of eight sampling sites in Southern Tasmania (Google map, 2019)

2.2.2 Sampling and processing

2.2.2.1 Macroinvertebrate sampling

The field sampling protocol and sample size were specifically modified and designed to be consistent with the AUSRIVAS sampling protocols and as such involved triplicate kick – samples (AUSRIVAS, 2014) at each site. The exception to the protocol was the preservative used for the samples; this study used ethanol to preserve animals as per a number of previous studies (Johnson, 2007; Leuven et al., 1985; Metzeling et al., 2003; Nerbonne and Vondracek, 2001; Rak et al., 2011; Rosenberg et al., 1998) in contrast to the AUSRIVAS protocol which uses the picking and identification of live samples rather than preserved.

At each site, samples were collected from the further most downstream station first, with sampling moving upstream to avoid unnecessarily disturbance of the macroinvertebrate community (Fries and Bowles, 2002) or 'contaminating' samples with drift invertebrates from upstream. Only riffle areas or sections with relatively fast flow were sampled as pools were often deep and inaccessible. The kick sampling approach used in this research followed the sampling protocols of Stark and Group (2001). A net (frame size - 0.25 m x 0.25 m with a 500 µm mesh) (Buss and Borges, 2008) was placed on the streambed and macroinvertebrates were kick-sampled in an upstream direction for 1 minute to dislodge substrate, vegetation, organic material and benthic invertebrates over a sampled distance of approximately 5 m.

Figure 2.6: Kick sample net (left) and kick sampling of macroinvertebrates (right)

2.2.2.2 Sample processing

Upon completion of sampling at each station, the kick net was removed from the water. The net was washed down to concentrate the collected material in the bottom of the net and contents transferred into a 500 ml plastic container labelled with the details of the site, to which 70% ethanol was added as a preservative.

In the laboratory, samples were rinsed with water on a 500 µm sieve to remove the ethanol and the retained material hand-picked of any large debris. Samples were sorted in a white illuminated tray to remove macroinvertebrates and all fauna was preserved in 70 ml plastic sample jars containing 70% ethanol. Following sorting animals were identified to the lowest practical taxonomic level for their respective groups (family, genus or species level) using taxonomic keys by Gooderham and Tsyrlin (2002), Williams (1980) and MDFRC (2009). Most of animals were identified to family level while some were classified to order, genus or species depending on how advanced the taxonomic keys were for respective invertebrate groups. Those different taxonomic levels were also used to calculate SIGNAL and biological indices. Total numbers were counted for each taxon. Reference specimens were checked for correct identification by Dr Toni Furlonge from Natural Research Management in the North (NRM North), Launceston, Tasmania.

2.2.3 Data collection and analysis

2.2.3.1 Data collection

The total number of individuals, total number of each taxonomic group and the number of taxa were counted to calculate a range of indices; including rank abundance, total abundance, taxa richness, Simpson diversity index, and the SIGNAL 2 index. These indices were used to compare changes in abundance and taxa richness, and community diversity between sites. Taxonomic levels were different between macroinvertebrate groups based on available classification keys. Therefore, taxonomic requirement of SIGNAL, biological indices and indicator species were also based on the different identified taxonomic levels in this study.

Total abundance is the total number of individuals present in each sample. Taxa richness is the total number of taxa identified in each sample. Rank abundance (relative abundance) is

the numbers of individuals in each taxon which were ranked from the highest to lowest at each site. Relative abundance of each taxon was then computed as a proportion of the total abundance of all taxa in a sample before these proportions will be then $\log_{10}$ transformed (Magurran, 2004).

The SIGNAL 2 index provides a measure (indication) of water quality at each station and consists of SIGNAL grades and the total number of taxa collected, and is a recommended MDFRC and AUSRIVAS metric for assessment of river condition (Chessman, 2003b; Gooderham and Tsyrlin, 2002). The SIGNAL 2 index can be calculated both with and without abundance weighting for each station (Chessman, 2003a). Each macroinvertebrate taxon was given a number from 1 to 10 according to its pollution intolerance or tolerance based on the MDFRC guidelines and Gooderham and Tsyrlin (2002); this is the SIGNAL 2 grade (Table 2.3). Taxonomic levels of SIGNAL 2 grades were also different between taxa; which was defined as lowest level of each taxon in the classification keys. Each station was then given a water quality rating based on SIGNAL 2 scores (Table 2.5) and the differences in water quality compared among stations and between sites, times and techniques. The SIGNAL 2 score without abundance weighting is the average of the SIGNAL 2 grades for macroinvertebrate taxa collected at each station. The weight factors are based on number of specimens of each taxon (Table 2.4) and is calculated for each taxon present at each station. The multiplication of SIGNAL 2 grade and weight factor was then conducted for each taxon in each station. The SIGNAL 2 score with abundance weighting for each station was computed using the following formula (Chessman, 2003a):

SIGNAL 2 Score = Total of (SIGNAL 2 Grade x weight factor) / total of weight fact

Table 2.3: SIGNAL 2 grade for pollution tolerance (MDFRC, 2009)

SIGNAL 2 Grade	Pollution Tolerance
10 – 8	indicates a greater sensitivity to pollution
7 – 5	indicates a sensitivity to pollution
4 – 3	indicates a tolerance to pollution
2 – 1	indicates a greater tolerance to pollution

Table 2.4: Weight factors based on number of specimens (Chessman, 2003a)

Number of specimens	Weight factor
1 – 2	1
3 – 5	2
6 – 10	3
11 – 20	4
> 20	5

Table 2.5: Water quality rating by SIGNAL 2 score (MDFRC, 2009)

Site score	Water Quality Rating
>6	healthy habitat
5 – 6	mild pollution
4 – 5	moderate pollution
<4	severe pollution

The Simpson index is a meaningful and effective diversity index (Magurran, 2004). This index was calculated for each station, and is expressed as

$$D = \sum p_i^2 = \sum \left(\frac{ni[ni-1]}{N[N-1]} \right)$$

where p_i = the fractional abundance of the i^{th} species

ni = the number of individuals in the i^{th} species

N = the total number of individuals

Diversity increases with a decrease in D value. Therefore, this study used the reciprocal 1/D of D which is widely used to express the Simpson index (Magurran, 2004).

2.2.3.2 Statistical analyses

Univariate analyses

Biological indices (total abundance, taxa richness and Simpson diversity index) were analysed individually with ANOVA in PERMANOVA to determine differences between region (fixed), season (fixed) and site (random). The PERMANOVA routine was used as the test is achieved via permutation which avoids violation of ANOVA assumptions and it is possible to interpret interaction terms involving random factors (Anderson et al., 2008). Data were square root transformed to minimise the impact of dominant values or outliers (Anderson et al., 2008) before the Euclidean distance matrices were calculated. The Pseudo-F ratio and P values (α=0.05) were obtained following permutations (N=9999) of the residuals under a reduced model. Monte Carlo P-values were used instead of permutational *P* values (P_{PERM}) because of low replication. Pair-wise *a posteriori* comparison tests were done to compare each pair of regions, seasons and sites.

Table 2.6: Factor models and the null hypotheses for comparisons

	Comparison	Factors	Null hypothesis
Model 1	Differences in macroinvertebrate community and biological indices between ten sites in two regions of Tasmania over two seasons	Region (fixed): North and South Season (fixed): Summer 2016, autumn 2016 Site (random) nested within Region x Season: ten sites	No differences between sites, regions and seasons
Model 2	Differences in macroinvertebrate community and biological indices between two sites in the North over four seasons in one year	Season (fixed): summer 2016, autumn 2016, winter 2016, spring 2016 Site (random): Brumby, Patricks	No differences between sites, seasons and site x season interaction
Model 3	Differences in macroinvertebrate community and biological indices between eight sites in the South in summer and autumn in two years	Season (fixed): Summer 2016, autumn 2016, winter 2017, autumn 2017 Site (random): eight sites	No differences between sites, seasons and site x season interaction

Multivariate analyses

Principal coordinates analysis (PCO) was used as a descriptive ordination technique to visualise separation of assemblages between region, season and site.

A permutational multivariate analysis of variances (PERMANOVA) was used to detect differences in macroinvertebrate community structure between region (fixed), season (fixed) and site (random). The PERMANOVA routine in PERMANOVA+ for Primer 6 (Anderson et al.,

2008) is based on any distance matrix, and uses permutation methods to calculate significance values. Data were square root transformed before Bray Curtis similarities were calculated. The PERMANOVA models for each hypothesis are described as Table 2.6. The Pseudo-F ratio and P values (α=0.05) were obtained following permutations (N=9999) of the residuals under a reduced mode. Monte Carlo P-values were used for Pair-wise *a posteriori* comparison tests to compare each pair of regions, seasons and sites.

2.3 Results

2.3.1 Differences in the macroinvertebrate community between streams in Tasmania over summer and autumn 2016

2.3.1.1 Total abundance, taxa richness and Simpson diversity index

There were no significant interactions between season and region for total abundance, taxa richness and Simpson diversity index (Table 2.7) indicating that the effect of region (North and South) on biological indices was independent of season (summer and autumn). Moreover, no significant differences in total abundance, taxa richness and diversity index were seen between the two seasons as well as the two regions. However, there were significant differences among sites within regions and seasons for all biological indices (Table 2.7). Figure 2.7 illustrates that the trend was different for each metric, described in the following text.

Within the South, total macroinvertebrate abundance of the Dee (South) was significantly higher than all other sites in both seasons (Table 2.7, Figure 2.7) while in the North, there were no significant differences between the Brumbys and St Patricks sites in both seasons (Figure 2.7 and Table 2.7). In autumn, significantly higher total abundance was recorded at the Derwent than at the Broad, Tyenna at Russell Falls, Florentine, Tyenna End, Styx and the

Ouse while the Tyenna End and the Russell Falls had significantly higher total abundance than the Broad and the Florentine. Although there was no significant difference in total abundance between the two seasons (Table 2.7), some sites had a trend for higher abundance in autumn (Dee and Derwent) (Figure 2.7). The highest total abundance occurred t at the Dee (about 1703 individuals) in autumn, which was approximately 6 – fold higher than that of the Broad (the lowest total abundance, approximately 207 individuals) followed by St Patricks (1017 individuals).

In the South, there was a significantly higher richness in the Derwent, Florentine and Russell Falls compared to the Broad and the Styx in summer, and significantly higher richness in the Ouse and the Derwent compared to Tyenna End, Broad and the Dee in autumn (Table 2.7). Within the South, there were no significant differences between the Brumbys and St Patricks in summer; however, Brumbys had a significantly lower taxa richness than the St Patricks in autumn. The highest taxa richness was recorded at the Derwent with approximately 26 taxa on average, followed by the Ouse (23 taxa). The lowest number of taxa occurred at the Broad and the Brumbys with about 15 taxa present (Figure 2.7).

There were similarities in Simpson diversity indices across two seasons at most of sites, except for the Styx, Ouse and the Derwent. In summer, the Derwent had a significantly higher Simpson's diversity index than all other sites while the Dee and the Florentine had significantly higher Simpson's indices than the Tyenna End. In autumn, Simpson's diversity index of the Ouse was significantly higher than the Broad, Florentine, Tyenna End and the Dee while the Derwent and Russell Falls were significantly higher than the Dee (Table 2.7). There were no significant differences in diversity indices between the Brumbys and St Patricks in both summer and autumn.

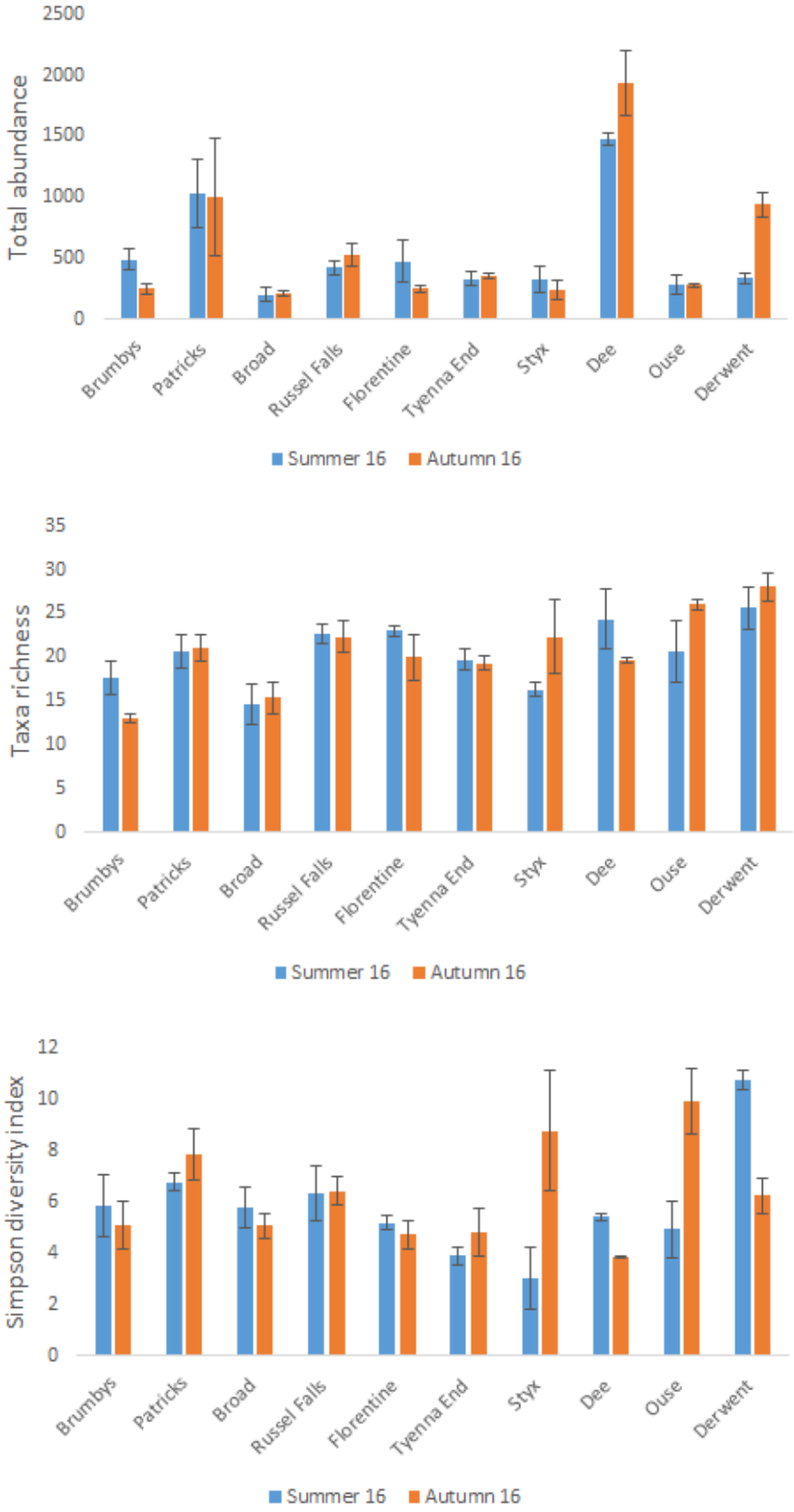


Figure 2.7: Mean (±SE; n=3 replicates) of three biological indices of macroinvertebrates in ten sites in two regions (North, South) over two seasons (summer, autumn). The first two sites are located in the North whereas the remaining sites are in the South.

Table 2.7: ANOVA testing the effect of season (Se), region (Re) and Site (Si) within ten sites on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced model. Pair-wise post hoc comparisons were done for site (season x region).

Source	Df	MS	P (MC)	Post hoc comparison	MS	P (MC)	Post hoc comparison
Transformation		Total abundance Square root			Taxa richness Square root		
Se	1	12.268	0.8257		0.0871	0.7095	
Re	1	106.31	0.5124		1.1917	0.1714	
SexRe	1	76.325	0.5830		0.3047	0.4854	
Si (SexRe)	16	238.71	0.0001	Summer: Dee ≠ others Autumn: Dee and Derwent ≠ others Tyenna and Russell Falls ≠ Florentine, Broad, Dee, Derwent	0.5938	0.0001	Summer: Broad, Styx ≠ Russell Falls, Florentine, Derwent Autumn: Brumbys ≠ St Patricks Ouse, Derwent ≠ Broad, Tyenna End, Dee
Residuals	40	22.189			0.1558		
Transformation		Simpson diversity index Square root					
Se	1	0.0512	0.7565				
Re	1	0.1197	0.6359				
SexRe	1	0.0275	0.8159				
Si (SexRe)	16	0.5294	0.0001	Summer: Derwent ≠ others Tyenna End ≠ Dee, Florentine Autumn: Ouse ≠ Broad, Florentine, Tyenna End, Dee Dee ≠ Russell Falls, Derwent			
Residuals	40	0.1036					

2.3.1.2 Signal 2 index

The data in Table 2.8 illustrates slight differences in SIGNAL 2 scores with and without weighting factor for the same sites. The SIGNAL scores for water quality ratings were similar at most sites with and without the weighting factor, except the Tyenna at Russell Falls and the Florentine 2 which resulted in *healthy habitat* with weighting factors and *mild pollution* without weighting factors. The two northern sites scored from *moderate pollution* to *mild pollution* while the Broad, Tyenna at Russell Falls, the Florentine of the South were scored

healthy habitat. The remaining sites of the South displayed signal scores of *moderate pollution* or *mild pollution*. Furthermore, water quality ratings for respective sites were similar between the two seasons.

Table 2.8: Water quality rating by site score in northern and southern Tasmania based on SIGNAL 2 with and without the abundance weightings applied (N: North, S: South, Sum: summer, Aut: autumn)

Sites	Season	Site Score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
N: Brumbys	Sum	4.62	moderate pollution	4.37	moderate pollution
N: St Patricks	Sum	5.50	mild pollution	5.80	mild pollution
S: Broad	Sum	6.31	healthy habitat	6.57	healthy habitat
S: Russel Falls	Sum	5.66	mild pollution	6.19	healthy habitat
S: Florentine	Sum	6.04	healthy habitat	6.28	healthy habitat
S: Tyenna End	Sum	4.96	moderate pollution	4.78	moderate pollution
S: Styx	Sum	5.39	mild pollution	5.43	mild pollution
S: Dee	Sum	4.97	moderate pollution	4.78	moderate pollution
S: Ouse	Sum	4.29	moderate pollution	4.11	moderate pollution
S: Derwent	Sum	5.78	mild pollution	5.32	mild pollution
N: Brumbys	Aut	5.00	moderate pollution	4.84	moderate pollution
N: St Patricks	Aut	5.84	moderate pollution	6.00	mild pollution
S: Broad	Aut	6.36	healthy habitat	6.59	healthy habitat
S: Russel Falls	Aut	6.33	healthy habitat	6.46	healthy habitat
S: Florentine	Aut	6.07	healthy habitat	6.61	healthy habitat
S: Tyenna End	Aut	5.54	mild pollution	5.58	mild pollution
S: Styx	Aut	5.55	mild pollution	5.58	mild pollution
S: Dee	Aut	4.84	moderate pollution	4.44	moderate pollution
S: Ouse	Aut	4.43	moderate pollution	4.18	moderate pollution
S: Derwent	Aut	5.45	mild pollution	5.28	mild pollution

2.3.1.3 Relative abundance

Dominance-diversity curves at all sites are flat in profile which indicates the community is rich in species diversity (Figure 2.8). The number of taxa ranged from 26 taxa (Brumbys) to 38 taxa (Dee) in summer and from 17 taxa (Brumbys) to 40 taxa (Derwent) in autumn.

The Brumbys site in autumn was dominated by Caenidae (mayflies) with a relative abundance of 0.26 (194 individuals/745 total individuals of whole community), followed by Hydropsychidae (caseless caddies), Hydrobiidae (mud snails) and Orthocladiinae (midges) being 0.197, 0.142 and 0.134 respectively. A total of 17 taxa occurred at this site, and the relative abundances of the other 13 taxa were less than 0.05. Similarly, the dominant taxa at the Broad was Baetidae (mayflies) being 0.36 (228 individuals/633 total individual of whole community), followed by Leptophlebiidae (mayflies) with 0.194 (123 individuals/633 total individual of whole community) with all other taxa being less than 0.095. In contrast, relative abundance at the Derwent in autumn ranged from 0.0003 to 0.2256 and total taxa sampled at this site was the highest (40 taxa). Moreover, Hydroptilidae (purse-case caddisflies), Hydrobiidae (mud snails) and Orthocladiinae (midges) were three taxa dominant at the Derwent site.

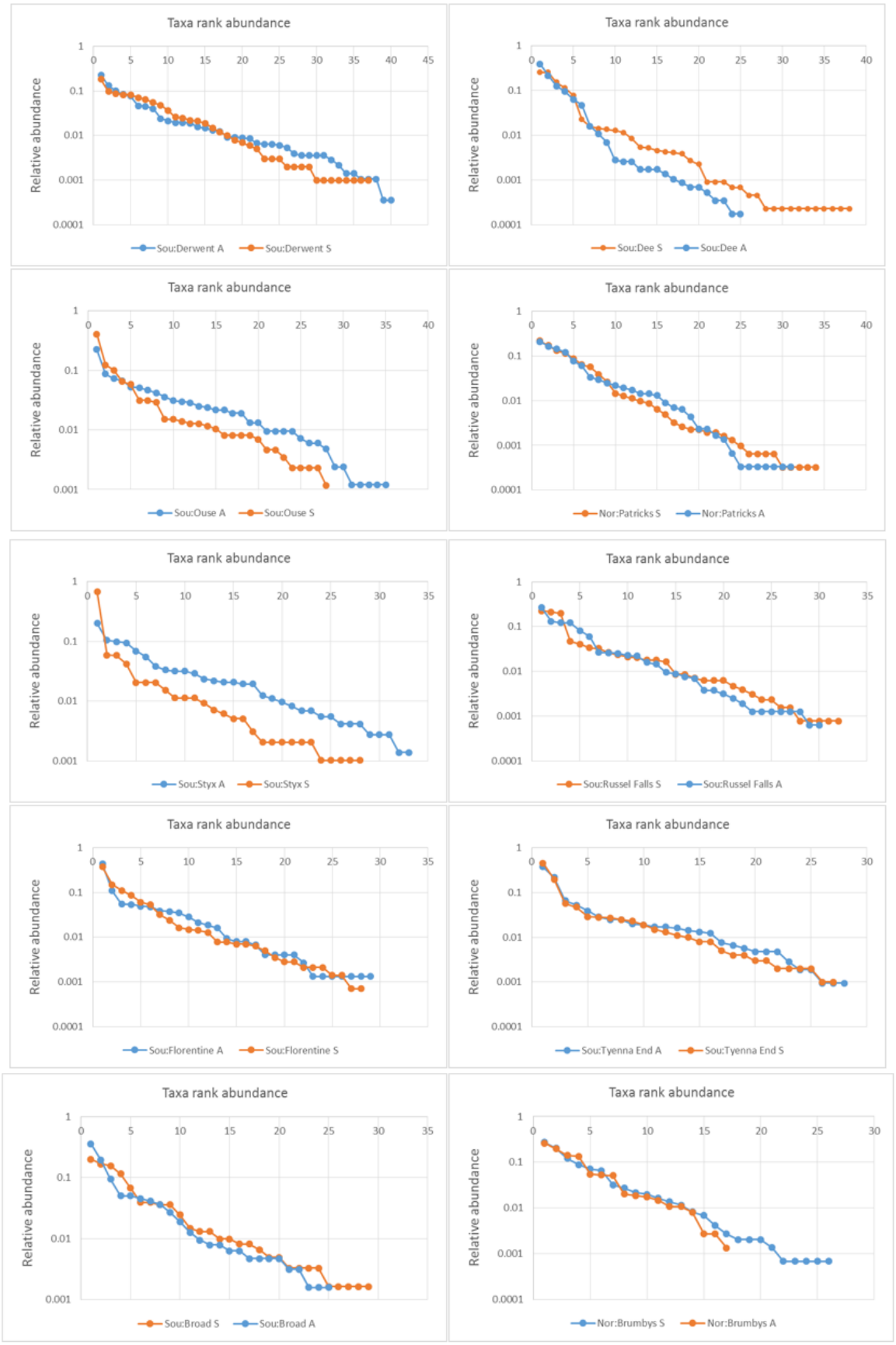


Figure 2.8: Rank abundance – plots for 10 sites in northern and southern Tasmania in summer and autumn 2016 (S: summer, A: autumn).

2.3.1.4 Macroinvertebrate community structure

The grouping of data between sites in two seasons is shown in the PCO plots (Figure 2.9). There is a clear separation of the Brumbys from the Patricks in the North as well as separation of the Broad, Russell Falls and the Florentine from all other sites in the South along PCO1. Moreover, PC01, which explains 31% of the total variation, separates the ten sites into three groups; group 1 (the Patricks, the Broad, the Russell Falls and the Florentine), group 2 (the Brumby, the Tyenna End, the Styx and the Derwent), and group 3 (the Dee and the Ouse). PCO2 explained 10.6% of the total variation and also separated the Dee from the Ouse while the other sites in groups 1 and 2 showed no obvious separation along PCO2. Furthermore, macroinvertebrate composition showed little difference between summer and autumn at all sites except for the Brumbys and the Derwent. Pair-wise tests showed that there were significant differences in macroinvertebrates between two seasons at the Brumbys and the Derwent (Pairwise PERMANOVA, P_{MC}=0.03 and P_{MC}=0.04 respectively) while macroinvertebrates did not differ significantly between summer and autumn at other sites.

There was also no significant interaction between season and region on the composition of the macroinvertebrate assemblages (PERMANOVA, $F_{1,40}$ = 0.28, P_{MC}>0.05). However, there were significant differences in macroinvertebrate communities between sites (PERMANOVA, $F_{16,40}$ = 8.71, P_{MC}=0.0001). Macroinvertebrate composition differed between all pairs of sites (Pairwise PERMANOVA, P<0.05) except for the Broad which did not differ significantly from the Russell Falls (P_{MC}=0.108), the Florentine (P_{MC}=0.1277) and the Styx (P_{MC}=0.1052).

Separation of sites along PCO1 reflected high positive vector loadings for Baetidae and Leptophlebiidae at Patricks, Broad, Russell Falls, Florentine and low loadings for a range of other taxa at these sites (Figure 2.9). As well as having lower loadings for Baetidae and

Leptophlebiidae, the separation of the Ouse along PCO2 also reflected high loadings for Hirudinae (leeches), *Cura* sp. (flatworms), Planorbidae (air-breathing freshwater snails), *Atyidae paratya* (freshwater shrimp) highlighting the high abundance of those taxa, while separation of Dee along the same axis reflected high loadings for Hydropsychidae, Hydrobiidae, Ceinidae (amphipods), Paramelitidae (amphipods) and Gyrinidae (whirligig beetles) (Figure 2.9).

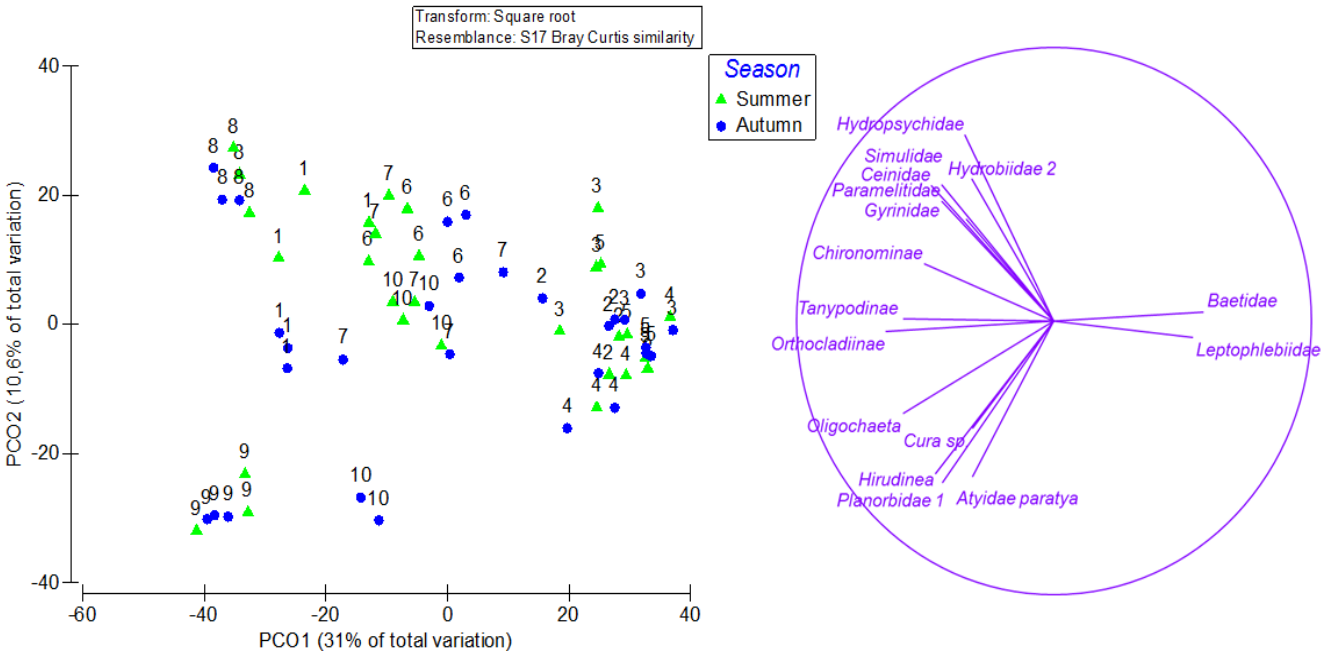


Figure 2.9: Two dimensional PCO plot for macroinvertebrate matrix fauna in North and South of Tasmania. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between stations (1: Brumbys (North), 2: St Patricks (North), 3: Broad (South), 4: Russell Falls (South), 5: Florentine (South), 6: Tyenna End (South), 7: Styx (South), 8: Dee (South), 9: Ouse (South) and 10: Derwent (South))

2.3.2 Similarities or differences between streams in Northern Tasmania over 4 seasons

2.3.2.1 Total abundance, taxa richness, Simpson diversity index

The total abundance of macroinvertebrates was significantly higher in the St Patricks than the Brumbys (Figure 2.10, Table 2.9). There was also a significant difference among seasons but the posthoc test revealed this was only a higher abundance in summer vs. winter. In the

Brumbys, macroinvertebrate abundance decreased from summer to winter, but rose slightly in spring whereas there was a slight decline in total abundance in the Patricks between summer and autumn before it dropped considerably in winter then increased slightly in spring.

There was a significant interaction between season and site for of macroinvertebrate taxa richness which reflected a significantly higher taxa richness in the Patrick compared to the Brumby in autumn, winter and spring (posthoc test, Table 2.9). Figure 2.10 illustrates that taxa richness of the Brumbys declined gradually over four seasons while taxa richness of Patricks increased slowly.

There were no significant differences in Simpson diversity index between seasons or sites (Table 2.9). Qualitatively, in the Brumbys the Simpson diversity index dropped slightly between summer and autumn before increasing in winter then decreasing in spring. In contrast, there was a slight increase in taxa richness of the Patricks between summer and autumn; this number then decreased gradually to spring.

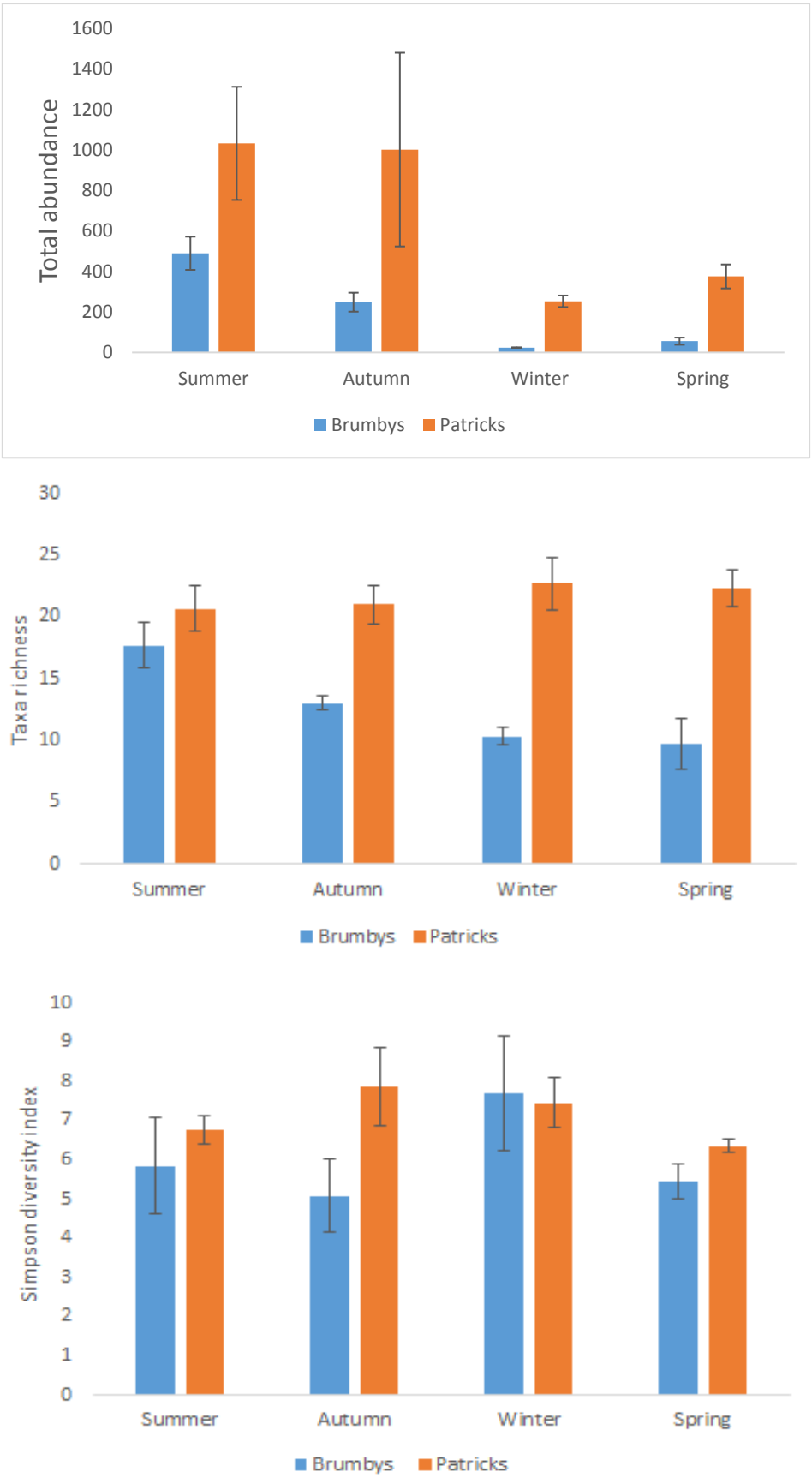


Figure 2.10: Mean (±SE; n=3 replicates) of three biological indices of macroinvertebrates at Brumbys and Patricks sites in the North over four seasons (summer, autumn, winter and spring).

Table 2.9: ANOVA testing the effect of season (Se), and Site (Si) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced mode. Pair-wise post hoc comparisons were done for significant effects of site, season, and site x season interaction.

Source	Df	MS	P (MC)	Post hoc comparison	MS	P (MC)	Post hoc comparison
		Total abundance			Taxa richness		
Transformation		Square root			Square root		
Se	3	357.37	0.0046	Summer > winter	0.2480	0.7171	
Si	1	815.79	0.0001	Brumby > Patricks in winter, spring	7.6143	0.001	
SexSi	3	6.2401	0.9014		0.5380	0.0189	Autumn, winter, spring: Brumby # Patricks
Residuals	16	32.724			0.1227		
		Simpson diversity index					
Transformation		Square root					
Se	3	0.1081	0.411				
Si	1	0.3306	0.0764				
SexSi	3	0.0840	0.4671				
Residuals	16	0.0943					

2.3.2.2 Signal 2 index

In relation to SIGNAL 2 scores, there were similarities in site scores between the two SIGNAL methods for all sites over four seasons. Table 2.10 illustrates that water quality rating were *moderate pollution* at the Brumbys over four seasons whereas *mild pollution* was scored at the Patricks over summer, autumn and winter; and the score was *healthy habitat* in spring.

Table 2.10: Water quality rating at three sites over four seasons based on SIGNAL 2 scores calculated with and without an abundance weighting factor

Site	Season	Site score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
Brumby	Sum	4.62	moderate pollution	4.37	moderate pollution
Patricks	Sum	5.50	mild pollution	5.80	mild pollution
Brumby	Aut	5.00	moderate pollution	4.84	moderate pollution
Patricks	Aut	5.84	mild pollution	6.00	mild pollution
Brumby	Win	4.44	moderate pollution	4.32	moderate pollution
Patricks	Win	5.97	mild pollution	5.95	mild pollution
Brumby	Spring	4.57	moderate pollution	4.80	moderate pollution
Patricks	Spring	6.03	healthy habitat	6.08	healthy habitat

2.3.2.3 Relative abundance

Dominance-diversity curves illustrate slight differences in taxa rank abundance between four sampling times at each site (Figure 2.11). The relative abundance of the St Patricks macroinvertebrate fauna was higher than that of the Brumbys site over the four seasons. The curves at Brumbys in autumn, winter and spring are steeper than summer as well as other curves of the St Patricks, indicating dominance by 1-2 taxa which contrasted with St Patricks. Higher relative abundance in summer and autumn compared to winter and spring was seen at Brumbys. In contrast, dominance-diversity curves of the St Patricks at four sample times are flatter in profile, highlighting a more even distribution of taxa abundance. Furthermore, relative abundance of the St Patricks in summer and autumn was slightly higher than those in winter and autumn.

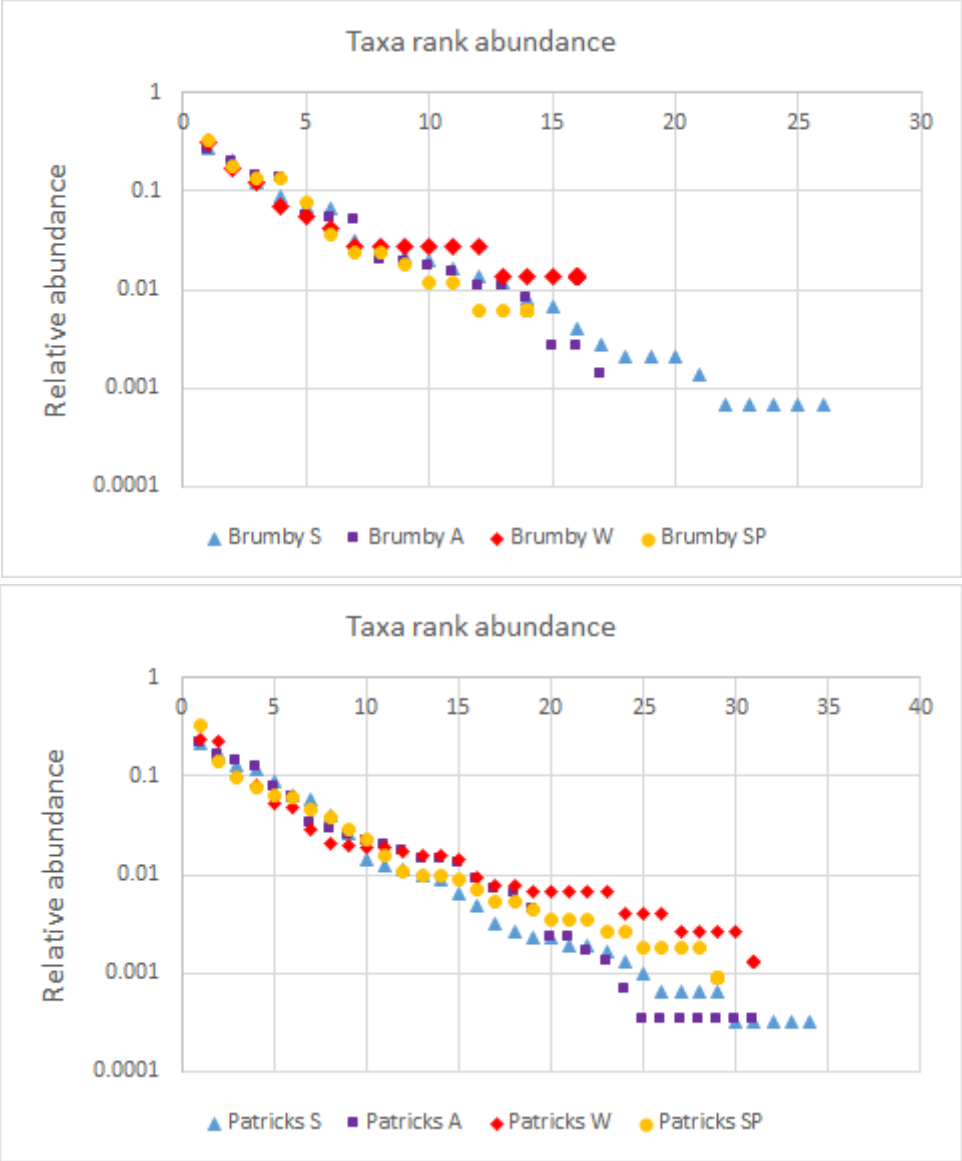


Figure 2.11: The dominance-diversity distribution for the Brumbys and St Patricks in Northern Tasmania over four seasons (order of sites in the graph determined randomly by Microsoft Excel) (S: summer, A: autumn, W: winter and SP: spring)

At Brumbys, Orthocladiinae (midges) (0.329), Simuliidae (black fly larvae) (0.18) and Caenidae (mayflies) (0.14) were dominant in spring while the dominant taxa in winter were Orthocladiinae (0.319) and Chironominae (midges) (0.17). In contrast, relative abundance at the Patricks in summer ranged from 0.00032 to 0.217 and the numbers of taxa sampled at this site was higher (34 taxa). Leptoceridae (caddisflies), Baetidae, Scirtidae (beetle larvae) were dominant at St Patricks in summer and their relative abundance were approximately

0.217 (673 individuals/3099 total individuals of whole community), 0.173 (536/3099) and 0.13 (404/3099) respectively.

2.3.2.4 Macroinvertebrate community structure

The PCO (Figure. 2.12) plot indicates the separation of the macroinvertebrate community in Brumbys from the St Patricks along PCO1 (which explain 43.3% of the variability in the dissimilarity matrix) as well as the separation of macroinvertebrates in summer and autumn from winter and spring along PCO2 (20.7% of the variability). Vector loadings along both axes indicated Hydropsychidae, Caenidae, Paramelitidae, Oligochaeta (aquatic worms) were associated with Brumbys in summer and autumn while Oniscigastridae (mayflies) was strongly associated with Brumbys in winter and spring. Vector loadings also indicated Leptophlebiidae, Baetidae, Scirtidae and Elmidae were strongly associated with St Patricks in summer and autumn while Simulidae, Hydrobiosidae, Gripopterygidae (stone flies) and Sphaeriidae (freshwater bivalve molluscs) were associated with St Patricks in winter and spring. The results of the PERMANOVA supported the patterns revealed by the PCO with a significant site x season interaction ($F_{3,16}$ = 6.61, P_{MC} = 0.0001). Pair-wise *posteriori* comparisons demonstrated that macroinvertebrate community structure was significantly different between sites each time as well as summer and autumn differing to winter and spring at both sites (pairwise PERMANOVA, $P<0.05$).

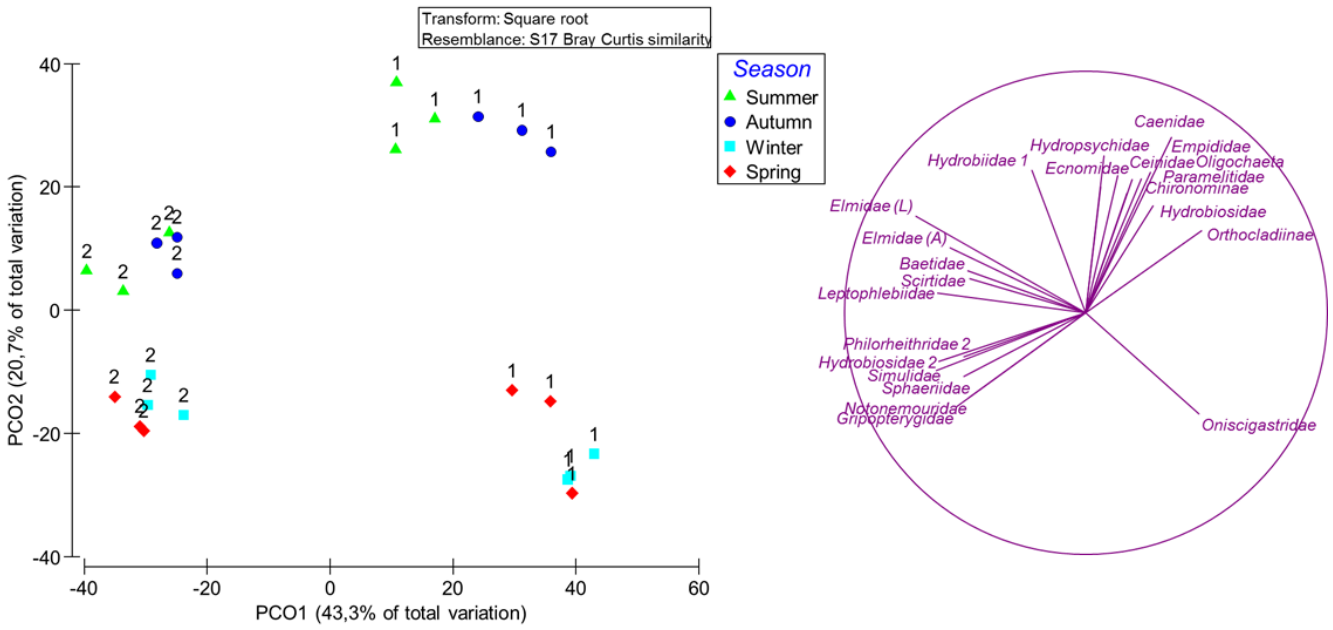


Figure 2.12: Two dimensional PCO plot for macroinvertebrate matrix fauna of two sites in the North. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between stations (1: Brumby, 2: Patricks)

2.3.3 Differences in macroinvertebrate community between streams in Derwent Catchment over summer and autumn in 2016 and 2017

2.3.3.1 Total abundance, taxa richness and Simpson diversity index

The total abundance of macroinvertebrates in the Derwent catchment differed with site and season (Figure 2.13, Table 2.11). The post hoc test revealed the Dee had a significantly higher abundance than all other sites in summer 16, autumn 16 and summer 17 (except the Tyenna End and the Ouse) (pairwise PERMANOVA, $P<0.05$). In addition, during summer 17 and autumn 17, the Broad had a significantly lower abundance than all other sites (except for the Ouse, Derwent in autumn 17) while in Autumn 16 the Derwent had a significantly higher abundance than all sites except the Dee (which was higher) (all pairwise PERMANOVA, $P<0.05$). There were temporal differences in total macroinvertebrate abundance at all sites except the Florentine and the Styx with significant differences between autumn 16 and

summer 17 at the Broad, the Tyenna End, the Ouse and the Derwent (pairwise PERMANOVA, $P<0.05$).

The taxa richness of macroinvertebrates in the Derwent catchment also differed with site and season (Figure 2.13, Table 2.11). The post hoc test revealed the taxa richness of the Broad and the Styx in summer 16 was significantly lower than the Russell Falls, the Florentine and the Derwent while the Broad, the Tyenna End and the Dee had a significantly higher taxa richness than the Ouse and the Derwent in autumn 16 (pairwise PERMANOVA, $P<0.05$). Furthermore, the Broad had a significantly lower taxa richness than all other sites in summer 17 and autumn 17 (except the Derwent) whereas there was significantly higher taxa richness at the Russell Falls and the Florentine compared to the Dee, Ouse and Derwent in autumn 17 (pairwise PERMANOVA, $P<0.05$). There were temporal differences in taxa richness of macroinvertebrates at the Styx, the Dee, the Ouse and the Derwent with significant differences between autumn 16 and summer 17 at the Dee, the Ouse and the Derwent (pairwise PERMANOVA, $P<0.05$). The highest taxa abundance was seen the Derwent. Conversely, the lowest taxa richness across the four seasons were recorded at the Broad.

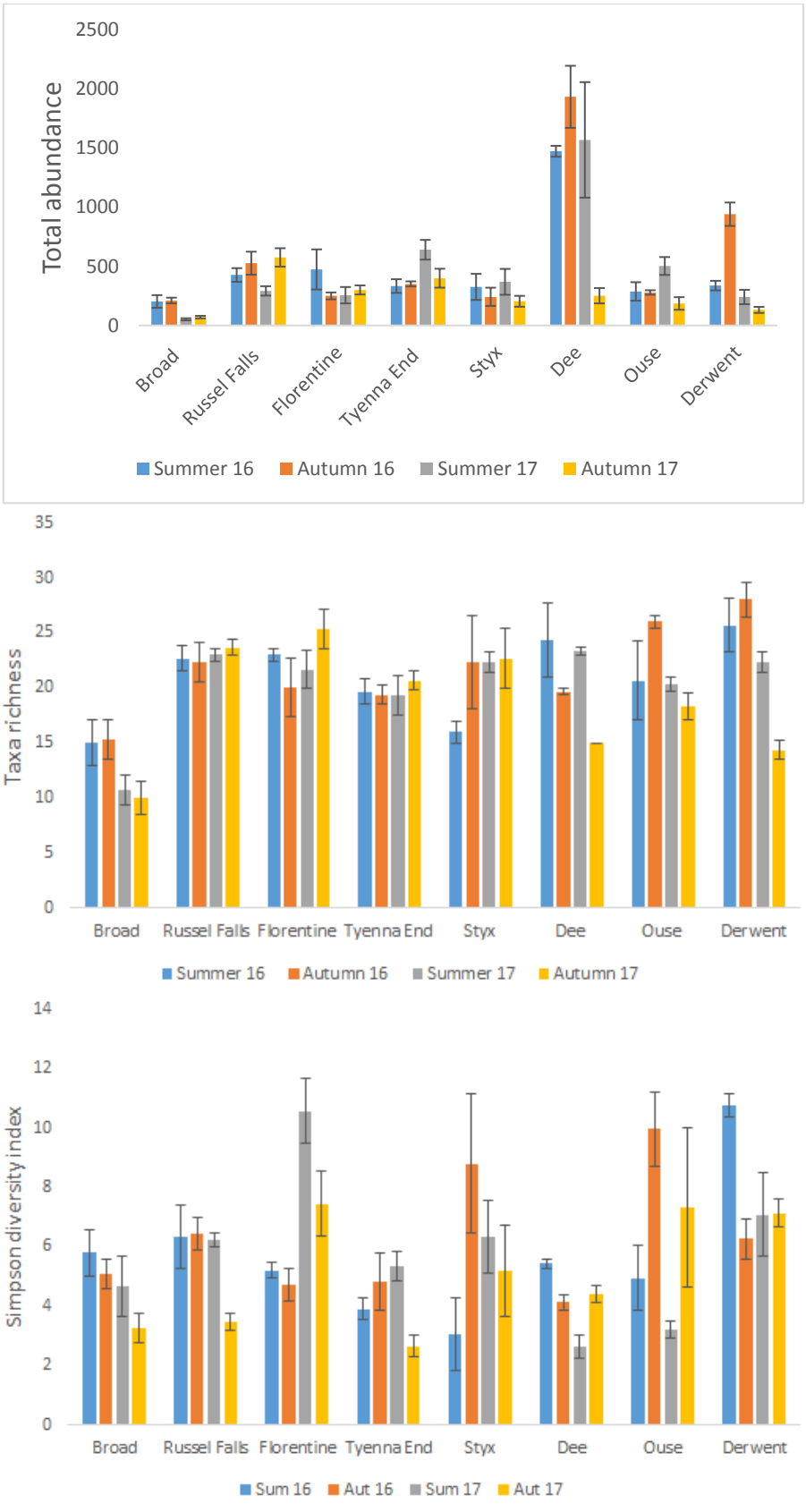


Figure 2.13: Mean (±SE; n=3 replicates) of three biological indices of macroinvertebrates of ten sites in the South over four seasons (summer 2016, autumn 2016, summer 2017 and autumn 2017).

Table 2.11: ANOVA testing the effect of season (Se), and Site (Si) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced mode. Pair-wise post hoc comparisons were done for site, season, and site x season interaction.

Source	Df	MS	P (MC)	Post hoc comparison	MS	P (MC)	Post hoc comparison
		Total abundance			Taxa richness		
Transformation		Square root			Square root		
Se	3	201.6	0.1281		0.4995	0.3398	
Si	7	529.34	0.0001		1.9115	0.0001	
SexSi	21	93.541	0.0001	- Sum 16: Dee ≠ others - Aut 16: Dee ≠ others Derwent ≠ others Russell Falls ≠ Broad, Florentine, Ouse Tyenna ≠ Broad - Sum 17: Broad ≠ others Dee ≠ others (except Tyenna End, Ouse) Tyenna End ≠ Russell Falls, Florentine, Derwent - Aut 17: Broad ≠ others (except Ouse, Derwent) Russell Falls ≠ others (except Tyenna End) Derwent ≠ Florentine, Tyenna End - Broad: sum16 ≠ sum17 aut 16 ≠ sum17, aut17 - Russell Falls: sum17 ≠ aut17 - Tyenna End: sum17 ≠ sum16, aut16 - Dee: aut17 ≠ sum16, aut16, sum17 - Ouse: sum17 ≠ aut16, aut17 - Derwent: sum16 ≠ aut16, aut17 aut16 ≠ sum17, aut17	0.4172	0.0001	- Sum 16: Broad, Styx ≠ Russell Falls, Florentine, Derwent - Aut 16: Ouse, Derwent ≠ Broad, Tyenna, Dee - Sum 17: Broad ≠ others Dee ≠ Ouse - Aut 17: Broad ≠ others (except Derwent) Russell Falls, Florentine ≠ Dee, Ouse, Derwent Tyenna End, Styx ≠ Dee, Derwent - Styx: sum16 ≠ sum17 - Dee: sum16, sum17 ≠ aut17 aut16 ≠ sum17, aut17 - Ouse: aut16 ≠ sum17, aut17 - Derwent: sum16, sum17 ≠ aut17 aut16 ≠ sum17, aut17
Residuals	64	13.726			0.1173		

Source	Df	MS	P (MC)	Post hoc comparison
		Simpson diversity index		
Transformation		Square root		
Se	3	0.2760	0.6731	
Si	7	0.8689	0.0001	

SexSi	71	0.526	0.0001	- Sum 16: Derwent ≠ others Tyenna ≠ Dee, Florentine - Aut 16: Ouse ≠ Tyenna End, Dee, Florentine, Broad Dee ≠ Russell Falls, Derwent - Sum 17: Florentine ≠ others (except Styx, Derwent) Dee ≠ Styx, Derwent, Tyenna End - Aut 17: Broad ≠ Tyenna End Florentine, Derwent ≠ Russell Falls, Dee, Broad - Broad: sum16 ≠ aut17 - Russell Falls: aut17 ≠ sum16, aut16, sum17 - Florentine: sum1, aut16 ≠ sum17 - Tyenna End: sum17 ≠ aut17 - Dee: sum16 ≠ aut16, sum17, aut17 sum17 ≠ aut16, aut17 - Ouse: sum16, sum17 ≠ aut16 - Derwent: sum16 ≠ aut16, aut17
Residuals	64	0.1128		

Similarly, the Simpson diversity index of macroinvertebrates differed with sites and season (pairwise PERMANOVA, $P<0.05$). The post hoc test showed that the diversity index of the Derwent was significantly higher than all other sites in summer 16 while the Tyenna End had a significantly lower diversity than the Dee and the Florentine (pairwise PERMANOVA, $P<0.05$). In autumn 16, the Ouse had significantly higher diversity than the Tyenna End, the Dee, the Florentine and the Broad whereas diversity index of the Dee was significant lower that the Russell Falls and the Derwent. In summer 17, macroinvertebrate diversity of the Florentine was significantly higher than all other sites, but not the Styx and the Derwent whilst the Dee had a significantly lower diversity than the Styx, the Tyenna End and the Derwent. In autumn 17, both the Florentine and the Derwent had a significantly higher Simpson diversity than the Russell Falls, the Dee and the Broad. There were temporal differences in the Simpson diversity index at all sites except the Styx with significant differences between autumn 16 and summer 17 at the Florentine, the Dee and the Ouse (pairwise PERMANOVA, $P<0.05$).

2.3.3.2 The SIGNAL 2 index

The SIGNAL 2 scores show some site differences with and without a weighting factor (Table 2.11). These two methods scored similar water quality ratings at eight sites over time. Most of the sites exhibit similar water quality ratings pre- (autumn 16) and post-flood (summer 17); except for the Broad, the Tyenna at Russell Falls and the Styx. While site scores at the Broad and the Russell Falls pre- and post-flood changed from *healthy habitat* to *mild pollution*; the Styx displayed site scores as *mild pollution* and *healthy habitat* prior to and after the flood respectively. In general, the Broad, the Russell Falls and the Florentine had healthier water quality rating compared to other sites. Water quality ratings at Tyenna End and the Derwent remained unchanged as *mild pollution* before and after the flood whereas site scores indicated *moderate pollution* for the Dee and the Ouse in both autumn 16 and summer 17.

Table 2.12: Water quality rating at eight sites over four seasons based on SIGNAL 2 scores calculated with and without an abundance weighting factor

Sites	Season	Site Score	Water quality rating	Site score **(weighting factor)**	Water quality rating (**weighting factor)**
Broad	Sum 16	6.31	healthy habitat	6.57	healthy habitat
Russell Falls	Sum 16	5.66	mild pollution	6.19	healthy habitat
Florentine	Sum 16	6.04	healthy habitat	6.28	healthy habitat
Tyenna End	Sum 16	4.96	moderate pollution	4.78	moderate pollution
Styx	Sum 16	5.39	mild pollution	5.43	mild pollution
Dee	Sum 16	4.97	moderate pollution	4.78	moderate pollution
Ouse	Sum 16	4.29	moderate pollution	4.11	moderate pollution
Derwent	Sum 16	5.78	mild pollution	5.32	mild pollution
Broad	Aut 16	6.36	healthy habitat	6.59	healthy habitat
Russell Falls	Aut 16	6.33	healthy habitat	6.46	healthy habitat
Florentine	Aut 16	6.07	healthy habitat	6.61	healthy habitat
Tyenna End	Aut 16	5.54	mild pollution	5.58	mild pollution
Styx	Aut 16	5.55	mild pollution	5.58	mild pollution
Dee	Aut 16	4.84	moderate pollution	4.44	moderate pollution
Ouse	Aut 16	4.43	moderate pollution	4.18	moderate pollution
Derwent	Aut 16	5.45	mild pollution	5.28	mild pollution
Broad	Sum 17	5.61	mild pollution	5.98	mild pollution
Russel Falls	Sum 17	5.68	mild pollution	5.86	mild pollution
Florentine	Sum 17	6.20	healthy habitat	6.44	healthy habitat
Tyenna End	Sum 17	5.74	mild pollution	5.97	mild pollution
Styx	Sum 17	6.22	healthy habitat	6.42	healthy habitat
Dee	Sum 17	5.13	mild pollution	5.03	mild pollution
Ouse	Sum 17	4.72	moderate pollution	4.82	moderate pollution
Derwent	Sum 17	5.76	mild pollution	5.67	mild pollution
Broad	Aut 17	6.94	healthy habitat	6.71	healthy habitat
Russel Falls	Aut 17	5.88	mild pollution	6.17	healthy habitat
Florentine	Aut 17	5.77	mild pollution	6.23	healthy habitat
Tyenna End	Aut 17	5.89	mild pollution	5.61	mild pollution
Styx	Aut 17	6.09	healthy habitat	6.11	healthy habitat
Dee	Aut 17	5.32	mild pollution	4.70	moderate pollution
Ouse	Aut 17	4.79	moderate pollution	4.47	moderate pollution
Derwent	Aut 17	5.16	mild pollution	5.34	mild pollution

2.3.3.3 Relative abundance

The dominance-diversity curves of all sites are flat in profile which indicates the communities are species rich (Figure 2.14). Moreover, each site generally has similar dominance-diversity curves over the four sampling times, illustrating that the taxa rank abundance is similar over time. Numbers of taxa ranged between 16 (Broad in autumn 17) and 40 taxa (Derwent in autumn 16) across all stations over time.

The Broad in autumn 2017 was dominated by Leptoceridae (case caddis) with a relative abundance of 0.44 (94 individuals/212 total individuals of community), followed by Leptophlebiidae (mayflies), being 0.33 (70 individuals/212 total individuals of community). In this site, only 16 taxa were found in autumn 2017 while there were 18 taxa in summer 2017 which was dominated by similar taxa: Leptophlebiidae (0.346), Leptoceridae (0.224) and Baetidae (0.09). Relative abundance at the Derwent in autumn 2016 ranged from 0.0003 to 0.2256 and the numbers of taxa sampled at this site was the highest (40 taxa). Hydroptilidae, Hydrobiidae, and Orthocladiinae were three most dominant taxa at this site.

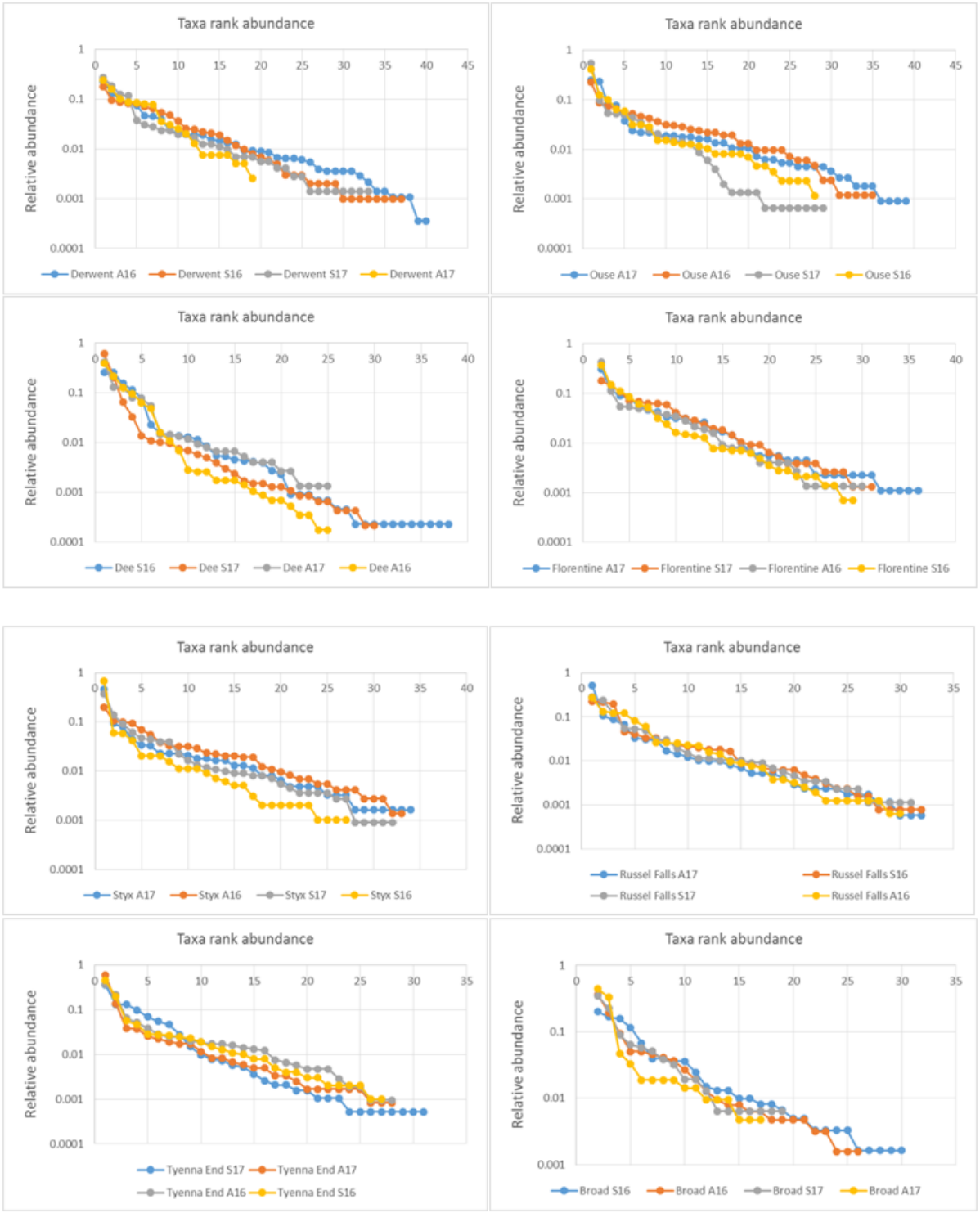


Figure 2.14: The dominance-diversity distribution for sites in Derwent Catchment over four sampling times (S16: summer 2016, A16: autumn 2016, S17: summer 2017, A17: autumn 2017)

2.3.3.4 Macroinvertebrate community structure

The PCO showed some evidence for separation of individual sites from others but also grouping together of some sites (Figure 2.15). PCO1 accounted for 27.8% of dissimilarity and suggested a slight separation of the group of the Tyenna End, the Styx, the Dee, the Ouse from the Russell Falls, the Florentine and the Derwent. PCO2 accounted for 11.3% of the variation and provided further separation of some sites for example, between the Dee and the Ouse; and the Broad from Russell Falls and the Florentine (Figure 2.15). The PERMANOVA revealed a significant season x site interaction ($F_{21,64}$ = 4.42, P_{MC} = 0.0001) and pairwise tests showed differences in macroinvertebrate communities between autumn 2016 (before the flood) and summer 2017 (after the flood) at all sites with the exception of the Styx; suggesting macroinvertebrate communities were changed by the impacts of the flood in winter 2016.

Vector loadings (Figure 2.15) for Planorbidae and Oligochaeta were positively correlated with two PCO axes and were strongly associated with the Ouse whereas Chironominae, Orthocladiinae, Tanyposinae and Hydropsychidae were positively correlated with PCO1 and highly associated with the Dee. Vector loadings for Leptophlebidae, Baetidae and *Eusthenia costalis* were highly associated with the Russell Falls and the Florentine.

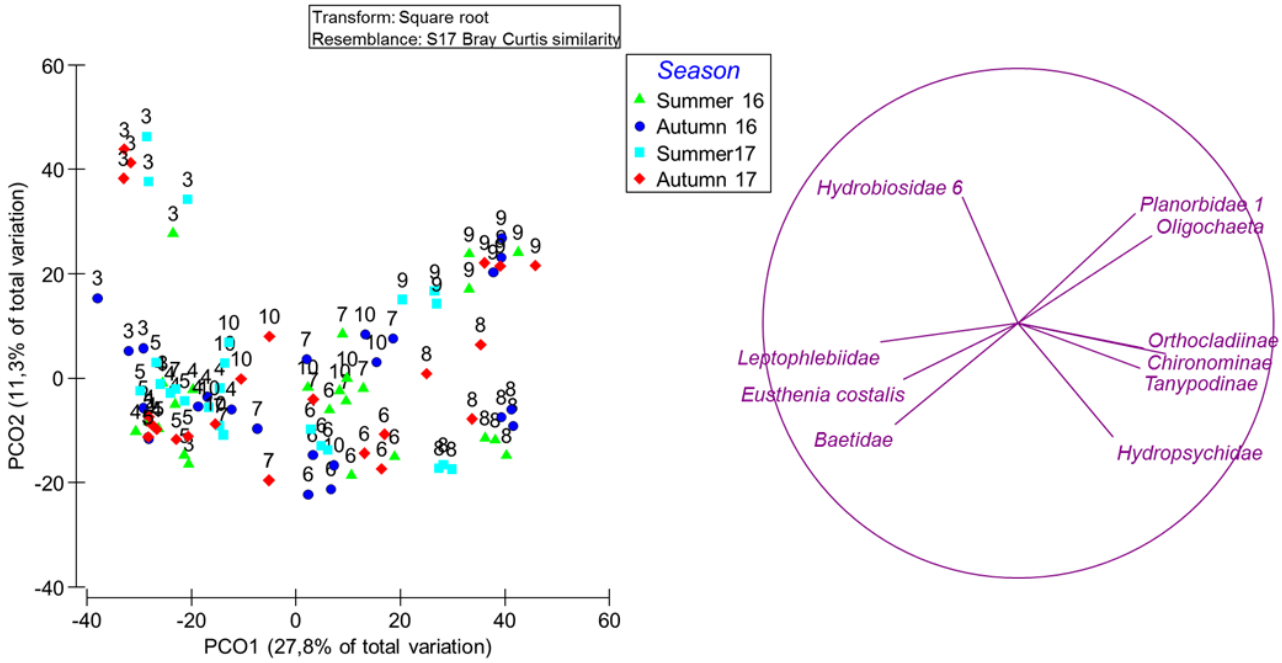


Figure 2.15: Two dimensional PCO plot for macroinvertebrate matrix fauna in South of Tasmania. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between stations. (3: Broad, 4: Russell Falls, 5: Florentine, 6: Tyenna End, 7: Styx, 8: Dee, 9: Ouse and 10: Derwent)

2.4 Discussion

2.4.1 Spatial and temporal comparisons

The comparison of biological patterns and macroinvertebrates assemblages indicated that the differences in the river communities were much more complex than simply a distinction between North and South. Macroinvertebrates reflect the changes of water resource, habitat (Gasith and Resh, 1999), and short-term hydrological (Extence et al., 1999). There were 4 distinct community groups identified (based on the PCO): group 1 including the St Patricks (North), Broad, Russell Falls and the Florentine (South); group 2 included the Brumbys (North), the Tyenna End, Styx and the Derwent (South); group 3 and 4 which are the Dee and the Ouse respectively. The St Patricks (North), the Broad, the Russell Falls and the Florentine; which are upland rivers with upper reaches of the streams flowing through forested areas with similar rocky and sandy substrate features, showed similarities in total abundance, taxa richness,

diversity index and macroinvertebrate community composition. Those streams were indicative of "*healthy habitat*" except for the St Patrick which was rated as "*mild pollution*".

Similarly, there were no significant differences in biological patterns and invertebrate assemblages between the Brumby (North), the Tyenna End, the Styx and the Derwent (South). Those streams were lowland rivers surrounded by grazing and agricultural areas; and all indicated "from *mild* to *moderate pollution*" for SIGNAL 2. In contrast, SIGNAL 2 indices indicated "*moderate pollution*" for the Dee and the Ouse. Both the Dee and the Ouse are very narrow lowland rivers surrounded by grazing, agricultural, urban and/or industrial areas with a low water depth and with rock and organic material overlaying rocks and pebbles. There was low diversity, but much higher total abundance and taxa richness at the Dee whereas the Ouse recorded a low diversity and abundance; but higher taxa richness. Azrina et al. (2006) reported that taxonomic richness and diversity index are higher in streams with better water quality, associated with unimpacted or unpolluted conditions, than with slightly polluted or polluted water quality. Moreover, there were very different macroinvertebrate communities between the Dee and the Ouse, as well as each differing significantly from other sites. Mykrä et al. (2007) explained that local environmental factors or catchment features (Richards et al., 1997; Roy et al., 2003a) cause changes in macroinvertebrate communities. Similarly, Macedo et al. (2014) suggested similarities or differences in streams assemblages depend on geophysical factors, land use, and anthropogenic impacts. Clenaghan et al. (1998) also reported that the variability in spatial and temporal patterns of invertebrate assemblages was affected by ecological, physical and chemical factors.

The observation of seasonal effects on aquatic fauna showed that there were changes in total abundance, taxa richness, diversity index and the macroinvertebrate community between

four seasons in two sites of Northern Tasmania. Within the North, there was a decrease in total abundance and taxa richness; and an increase in diversity index at the Brumbys in winter whereas there was a considerable decrease in total abundance but a slight decrease in diversity index at the St Patricks and a slight increase in taxa richness at the St Patrick in winter. Robinson et al. (2004) explained that macroinvertebrate communities respond immediately to floods often caused by a decrease in abundance; which is likely due to macroinvertebrate being swept downstream in the flood (Imbert and Perry, 2000; Perry and Perry, 1986). The SIGNAL 2 index indicated no changes in water quality rating at both sites prior to (autumn 2016) and post-flood (winter 2016), which were "*moderate pollution*" and "*mild pollution*" at the Brumbys and the St Patricks sites respectively. Those results suggest that there were minor impacts of the flood on macroinvertebrates at the Brumbys and St Patricks sites. However, multivariate analysis showed that macroinvertebrate composition in autumn 2016 at two sites were different from in winter 2016 although there were similarities in macroinvertebrate structure in summer and autumn as well as in winter and spring at both sites. This indicated changes in the community compositions after the flood despite no changes in water quality ratings at those sites. This might be because some new components of community after the flood still had similar signal grades which resulted in similar site scores between before and after the flood.

Similarly, impacts of the flood on total abundance, taxa richness, diversity index occurred at some sites in the South; including the Broad, the Florentine, the Tyenna End, the Dee, the Ouse, the Derwent. However, macroinvertebrate community structure at all southern sites (except for the Styx) differed significantly pre- (autumn 2016) and post-flood (summer 2017). Macroinvertebrates assemblages are strongly influenced by flow (Dewson et al., 2007; Wood

and Armitage, 1999; Wright and Berrie, 1987) although the time-scale of assemblage changes in relation to floods can vary. For example, changes in communities can occur over periods of years rather than months (Robinson et al. (2003) but invertebrates can recover to similar levels as the pre-flood status within 132 days, even though there was a decrease in density, biomass and taxa richness of macroinvertebrates after a major flood (Scrimgeour et al. (1988). This can explain for our results of significant differences in macroinvertebrate composition within observed sites after flood in Derwent Catchment as the samplings were taken more than 4 months post flood, thus the community may be recovering at the sampling time; explaining why the SIGNAL 2 index did not change much at all sites before and after the flood. This might be because new components of community after the flood still had similar signal grades which resulted in similar site scores like in the North or the community only changed the abundance of each single taxa which also resulted in similar site scores. SIGNAL 2 indicated that stream quality ratings at some sites (the Florentine, the Tyenna End, the Ouse and the Derwent) were similar before and after the flood. Water quality ratings (SIGNAL 2) at the Broad, the Russell Falls decreased after the flood while there were increases in water quality ratings at the Styx and the Dee after the flood.

2.4.2 Methodological comparison

Biological metrics (total abundance, taxa richness and diversity) illustrate general information about ten sites in Tasmania. However, they did not determine clear differences in macroinvertebrate communities within region and between sites over seasons. PERMANOVA and PCO procedures did show dissimilarity between sites over seasons, and corresponding invertebrate indicators of sites across that separation. PERMANOVA and PCO (Figure 2.9, 12 & 15) also suggested that Chironomidae, Hirudinae, Planorbidae, Physidae, *Cura sp.*, Ceinidae

and Paramelitidae and Oligochaeta were indicators for sites rated as "*mild* to *moderate pollution*" (lowland rivers) while Scirtidae, Hydrobiosidae, Leptophlebiidae, *Eusthenia costalis*, and Elmidae were indicative of cleaner sites (highland rivers) and decreased the abundance at impacted sites. Similarly a higher density and biomass of Oligochaeta and Chironomidae occurred when water quality became polluted due to anthropogenic activities such as land clearing and river regulation (Chessman, 1995). Moreover, Czerniawska-Kusza (2005) found a considerable decrease or disappearance in the number of pollution intolerant taxa; including caddisflies (Trichoptera - Limnephilidae, Leptoceridae, Polycentropodidae, Hydropsychidae), mayflies (Ephemeroptera - Heptageniidae, Ephemerellidae, Ephemeridae, Baetidae, Caenidae) at polluted areas.

Moreover, SIGNAL 2 could determine distinct differences in pollution levels for each site. We suggest that the combination of PERMANOVA and SIGNAL 2 can distinguish community and stream quality differences between sites. This combination of methods not only demonstrated differences in macroinvertebrate composition, but it also showed differences in stream quality, which has been recommended by some previous studies. This is similar to the suggestion of Magurran (2004) which recommended that biological indices such as richness and Shannon diversity showed slight differences between different habitats such as low-flow and high-flow areas while PERMANOVA (and ordination approaches such as MDS) was a robust method to assess statistical differences in community between areas. Similarly, Marchetti et al. (2011) recommended that PERMANOVA and MDS were effective in detecting changes to assemblage composition while the biodiversity metrics were not.

Nonetheless, Chessman (1995) showed the abundance and diversity play an important role in illustrating ecosystem health, function and balance. Therefore, the observation of those

may remain necessary when we conduct studies on the effects of effluents on macroinvertebrate as well as receiving water. Edgar and Barrett (2002) and Winberg et al. (2007) agreed that multiple ecological measures such as abundance, taxonomic richness and diversity index could describe a wide range of ecological heterogeneity at multiple spatial scales.

In general, SIGNAL 2 can be a rapid assessment tool using macroinvertebrates to determine water quality caused by aquaculture operations. Multiple ecological measures (abundance, taxonomic richness and diversity index) combined with PERMANOVA, PCO and SIGNAL 2 can determine many key aspects of sites and stations. However, the methods used in this study were time consuming and therefore finding a quick method would be ideal, hence the interest in indicator species.

2.5 Conclusion

In general, this study has shown variability in the macroinvertebrate communities inhabiting streams in Tasmania. Macroinvertebrate community composition was not significantly different between the two regions (North and South) with the two sites in the north being more similar to sites in the south than to each other. Differences in macroinvertebrate communities between sites presumably reflect differences in catchment habitat (catchment land use, catchment size and flow rates) and substrate characteristics. The Patricks (North), the Broad (South), the Tyenna at Russell Falls (South) and the Florentine (South) which were all upland streams with a sandy and rocky substrate and surrounded by forests; had similarities in macroinvertebrate communities. The Brumbys (North), the Tyenna End (South), the Styx (South) and the Derwent (South) are located in a lowland grazing and agricultural areas; were also similar in community composition of benthos. The Dee and the Ouse in the

South are small lowland streams with very low water depth, and organic material overlaying rocky substrate in grazing, agricultural, urban and industrial area, and high anthropogenic impacts. Both these sites showed significant differences in invertebrate community from other sites.

The investigation of major flood in 2016 showed that there were differences in macroinvertebrate community composition pre and post flood at all sites (except the Styx) in the South of Tasmania. In the North, there were season changes in macroinvertebrate assemblages at the two sites (the Brumbys and the St Patricks). Macroinvertebrate communities were similar in summer and autumn as well as in winter and spring; but were different between prior (autumn) and post flood (winter). Moreover, SIGNAL 2 without a weighting factor was likely to be a robust method for a rapid assessment of hatchery water quality as they highlighted water quality ratings basing on types of macroinvertebrates present without counting their abundance. In contrast, multivariate analyses such as PERMANOVA and PCO combined with SIGNAL 2 index were an effective tool for formal studies that can determine differences in macroinvertebrate community, indicator species and water quality between different sites.

This chapter investigated the macroinvertebrate fauna at ten sites in northern and southern Tasmania which suggested river characteristics and location relative to agriculture and other anthropogenic activities influenced faunal assemblages. The study demonstrated (based on invertebrate assemblages and scores implying water quality levels) that some rivers were “cleaner” than others and that community composition was influenced by season and extreme events such as a major flood. These descriptions will again be used in the following chapters where the findings will be used as a baseline with which to compare aquaculture

farm outlets and the impact on the fauna. While a number of sites were initially analysed together in this chapter to gain a broad comparative perspective, more intricate analyses will be undertaken in chapters 3-5.

3 Chapter 3: Do stream macroinvertebrate communities differ among streams with vs. without farms?

Abstract

Macroinvertebrate assemblages were sampled at 14 sites in Tasmania in April 2016 to compare between ten non-aquaculture sites of Brumbys Creek, St Patricks River, Florentine, Russell Falls, Broad, Tyenna End, Styx, Dee, Ouse and Derwent River with four aquaculture sites of Brumbys Creek, St Patricks River Florentine and Russell Falls. Macroinvertebrate communities responded differently depending on their stream geomorphology, natural habitat, and farming conditions. Farm effluents influenced total abundance and taxa richness but not Simpson's diversity; with impacted sites having a higher total abundance due to a high number of dominant species (mostly pollution tolerant taxa) and a lower SIGNAL 2 index. Multivariate analysis illustrated four site groupings having similar community structure and indicating different level of impact. The group containing St Patricks (North), Broad (South), Russell Falls (South) and Florentine (South) had a community structure indicative of clean water; the group containing Styx (South) and Tyenna End (South) were indicative of mild to moderate pollution; the group containing Brumbys (North), Derwent (South), Dee (South) and Ouse (South) were indicative of moderate pollution; while the group containing four sites downstream of farm outfalls at Brumbys, Patricks, Russell Fall and Florentine had a community structure which indicated moderate to severe pollution. Taxa including Oligochaeta, Planorbidae, *Physa acuta,* Hirudinea, Sphaeriidae, and Chironomidae were indicators for polluted sites while *Eusthenia costalis* and Baetidae were indicative of clean sites. Furthermore, Oligochaeta played a key role in the differentiation of the upstream and downstream communities and this taxon was very abundant at the farm outfall sites.

The study has found 1) that there was evidence of farming effects at the downstream sites but this differed between rivers. 2) differences in the inherent condition of the rivers has the potential to affect their ability to assimilate farm inputs (and consequently, some rivers may be more resilient) 3) that whilst simple indices such as relative abundance, total abundance, taxa richness and Simpson diversity index may provide an indication of effects, they were unable to detect subtle changes and multivariate analyses of community structure were the most useful approach to obtain a clear gradient of effect. 4) that SIGNAL 2 did identify clear indicators of impact; and applying SIGNAL 2 and indicators as a quick approach to assess and monitor aquaculture farms is recommended.

3.1 Introduction

Aquaculture has grown worldwide in recent years, and the demand for seafood suggests that this growth will continue (Bostock et al., 2010). However, intensive fish production has the potential for negative effects on the surrounding environment (Bostock et al., 2010; Naylor et al., 2000), specifically on water quality (Wu et al., 1994). Impacts are predominantly due to nutrient and solid waste discharge from fish farms (Amirkolaie, 2011; Aure and Stigebrandt, 1990; Bostock et al., 2010; Gowen and Bradbury, 1987; Kelly et al., 1996) generated by uneaten feeds, fish excretion, faecal material, soluble metabolites, medications and pesticides (Bergheim and Selmer-Olsen, 1978; Carroll et al., 2003a; Kelly et al., 1996; Kendra, 1991; Wu, 1995). These effects can result in a reduction in ecosystem health and biodiversity (Bostock et al., 2010). Furthermore, if there are multiple nearby farms, or where the farming activity is particularly intensive or unregulated, there is potential for aquaculture activities to have an adverse effect on the fish farms themselves (Silvert, 1992).

Although the majority of fish produced worldwide is grown in ponds, relatively few studies have reported on the environmental impacts of pond culture with most studies focussing on cage aquaculture, partly because this is often a more intensive form of aquaculture particularly in developed countries. There has also been considerable media attention focused on the rapid expansion of salmon farming. In, Australia the salmonid annual production was 53 t in 1986 – 1987 (Irvin et al., 2018) and reached approximately 48,614 t in 2014–2015; on which Tasmania accounted the largest salmonid production volume and value over the period from 2004 – 2005 to 2014 – 2015 (Savage, 2016). In addition, although much of the research has focused on marine systems, fish farming can also have negative impacts freshwater aquaculture farms, but considerably less work has been undertaken to establish their impacts and develop reliable indicators and management practices for freshwater systems. The following sections outline the impacts of aquaculture and some of the mitigation strategies that have been employed to reduce or minimise impacts in freshwater systems.

In mainland Australia, the first published research on impacts of farms on stream macroinvertebrates was conducted by Webb (2012a), who examined the impacts of five trout farms in the Goulburn Valley, Victoria by investigating differences in invertebrate assemblages between upstream and downstream stations. This study found that the higher production intensity the farm had the greater negative impacts on ecology and stream invertebrates. Other studies on macroinvertebrate assemblages have focused on biological health of rivers (Chessman, 1995) and highlighted pollution impacts and other anthropogenic disturbances in rivers and streams (Chessman and McEvoy, 1997).

In Tasmania, research has mainly concentrated on exploring impacts of marine cage farming operations rather than those of freshwater hatchery farms. In the marine environment,

macroinvertebrates have been used to assess environmental condition influenced by marine salmonid aquaculture (Edgar et al., 2005) or catchment activity in estuaries (Edgar and Barrett, 2000). Surveys using macroinvertebrates further analysed the health of estuaries (Edgar et al., 1999), intertidal areas (Spruzen et al., 2008), protected areas (Barrett et al., 2009), and the response of infaunal macrobenthic communities under salmonid sea-cages to organic enrichment (Ritz et al., 1989) such as assessing the rate of macrobenthic recovery to establish appropriate monitoring and management approaches (Macleod et al., 2004).

In Tasmania, the salmon industry has been required to monitor its broader scale impacts in key farming areas for a number of years. A Broadscale Environmental Monitoring Program (BEMP) has been in place in the D'Entrecasteaux Channel and Huon Estuary since 2009, and an evaluation of this data was conducted from 2009 to 2012 to assess both farming and broader effects on water and sediment quality (Ross and Macleod, 2006). Sediment components with the BEMP included biota (infauna) and sediment chemistry (redox, stable isotopes, particle size and sulphide), whilst water quality parameters were made up of dissolved oxygen, temperature, salinity, DO saturation, nutrient (ammonia, nitrate, phosphate, silicon, total nitrogen, total phosphorous), and phytoplankton (HPLC cell counts, chlorophyll a, abundance/diversity). There have been two other monitoring programs in Tasmanian catchments, these were undertaken as part of the Derwent Estuary Program (DEP, 2009) and the Tamar Estuary and Esk Rivers program (TEER, 2011); and have focused on water quality and physico - chemical parameters, not on macroinvertebrates. The DEP monitoring program is focused on assessment of restoration potential and the recovery impact process and to promote the condition of the Derwent Estuary. In recent years this has expanded to include monitoring in the Derwent freshwater catchment (dominated by agriculture, forestry,

hydropower generation and fish hatcheries) and ambient water quality monitoring. For the ambient water quality monitoring of the DEP program, water quality parameters have been collected on a monthly basis since 2009 at up to 28 sites throughout the Derwent estuary by Norske Skog Boyer, Nyrstar Hobart and the Tasmanian State Government. The parameters assessed include temperature, salinity, pH, dissolved oxygen, turbidity, true colour, nitrate-N, nitrite- N, ammonia-N, total nitrogen, total phosphorus, total suspended solids, total and dissolved nutrients, organic carbon, zinc, and chlorophyll *a*; and have been monitored to manage and control algal problems. The Derwent Catchment Review project (Eriksen et al., 2011) was initiated to manage water quality and quantity in the greater Derwent catchment. This project concentrated on physico-chemical parameters: temperature, dissolved oxygen, pH, conductivity, nitrate-N, nitrite- N, ammonia-N, total nitrogen, total phosphorous, filterable reactive phosphate, turbidity, conductivity, total suspended solids , chlorophyll a, blue-green algae, which were sampled in the Upper Derwent, the Lower Derwent, Western inflows to the Derwent River, Eastern inflows to the Derwent, Derwent below Meadowbank to New Norfolk Bridge to assess their response to seasonal changes.

Whilst establishing water quality to ensure environmental sustainability is important, there has also been a growing concern about the quality of drinking water in Southern Tasmania. The Derwent River catchment supplies 60% drinking water for Hobart city and neighbouring towns (Eriksen et al., 2011), and there were a number of reports of adverse taste and odour issues in water from the Derwent (Lohberger, 2015; Luttrell, 2015). Although TasWater has regulated water quality, they has more regularly monitored water quality at a range of sites in Derwent catchment since the issues were identified (Luttrell, 2015). More recently, there have been discussions between Natural Resource Management (NRM South), NRM North,

Derwent Estuary Program, Southern Water, Hydro Tasmania, Department of Primary Industries, Parks, Water and Environment (DPIPWE) regarding how best to implement and manage a monitoring program to support environmental improvement (Eriksen et al., 2011).

The TEER aims to similarly maintain and enhance the Tamar Estuary and Esk Rivers (TEER, 2011). Water quality sampling has been undertaken monthly for the last 10 years in conjunction with the Environmental Protection Authority (EPA) Tasmania, with the aim of supporting management to maintain or improve water quality. In the TEER program samples are collected for general water condition (i.e. chlorophyll a, total phosphorus, total nitrogen, nephelometric turbidity units, and dissolved oxygen), recreational water quality (i.e. bacteriological counts) and also a range of key metal contaminants of interest in this system (i.e. copper, lead, zinc, cadmium, aluminium and arsenic), with local water quality targets established for many parameters. The sampling results are used to calculate an integrated Ecosystem Health Index (EHI) which provides a measure that managers can use to protect and improve vulnerable ecosystems in the Tamar. However, the ability to link these measures back to actual impact sources and how these can be managed has not yet been tested.

Macroinvertebrates are an important inclusion in impact studies as they have been used widely to examine environmental effects from disturbance (Azrina et al., 2006; Camargo, 1994; Chessman, 1995; Goodnight, 1973; Metcalfe, 1989; Slooff, 1983). However, most studies on freshwater systems have not really focused on particular sources of contamination, such as aquaculture, but rather on general river health (Hardie et al., 2012; Humphries et al., 1996). This is certainly true of the AUSRIVAS and Tasmanian river health program. The relationship between macroinvertebrates and environmental variables has been demonstrated in marine/ estuarine systems (Edgar and Barrett, 2002), and Humphries (1996)

has shown that there are similar relationships between macroinvertebrates and aquatic macrophytes, and between macroinvertebrates and water levels in a lowland Tasmanian river. While the present study could use water quality to assess impact of farms (and does look at the relationship between invertebrates and water quality parameters in Chapter 5) Kaushik and Cowey (1991) noted that water quality will fluctuate in response to daily husbandry activities such as feeding and cleaning, suggesting water quality parameters would not be appropriate for the long-term effects of any environmental changes. Hardie et al. (2012) examined a number of Tasmanian rivers to observe the response of macroinvertebrate communities to low flows, while Furlonge et al. (2015) tested the response of macroinvertebrate richness and assemblages from 66 different protected wetlands around Tasmania. Macroinvertebrates have also been used to observe changes in their communities in relation to catchment forest operations (Smith et al., 2009). Norris et al. (1982) examined the impacts of mine effluents on benthic macroinvertebrates in the South Esk River, north-eastern Tasmania while Humphries et al. (1996) conducted research on the macrobenthic assemblages of littoral habitats in the Macquarie and Mersey rivers to facilitate regulatory river management. Previous studies suggested that macronvertebrates are a good indicator of impact, even of particular impacts such as organic enrichment, that they can establish an impact gradient and therefore that they can be useful in terms of determining causality. Although there has been some work done for government agencies and industry (a requirement under the Tasmanian EPA's Development Application (DA) Conditions), it is commercial in confidence, and to my knowledge there is no published research investigating the impacts of aquaculture on stream invertebrates in receiving water in Tasmania.

Tasmanian state government authorities, such as the Environmental Protection Authority (EPA), are anxious to understand and put in place risk appropriate management strategies for the discharge of nutrient rich water from land-based aquaculture systems given the intended expansion plans of the salmon industry in Tasmania. Current compliance requirements within the Environmental Management and Pollution Control Act 1994 and the State Policy on Water Quality Management 1997 (SPWQM), state that aquaculture farms implement monitoring programs for existing farms, proposed new developments or upgrades to existing activities. This monitoring includes measurement of physical and chemical water quality and sediment quality as well as biological assessment specifically AUSRIVAS macroinvertebrate data, in order to establish both baseline conditions and potential impact areas. AUSRIVAS uses macroinvertebrates to define ecological health of rivers and recommends sampling in spring and autumn to account for any seasonal variation in the macroinvertebrate fauna. As requirements from the EPA for hatchery farms, a number of different approaches can be used to analyse and assess the response of macroinvertebrates to the farm discharge: AUSRIVAS, SIGNAL 2, EPT, family richness and total abundance; which can be indicative of level of pollution as well as community diversity. The AUSRIVAS model observes taxonomic composition in order to compare with the expected composition at unimpacted sites while SIGNAL 2 is an index which observes stream water quality through the community present, their abundance and their signal grades. Taxa richness and total abundance can only illustrate the diversity of the community but cannot notice any sources of pollution. Ephemeroptera, Plecoptera and Trichoptera families (EPT) were used as pollution indicators because they are intolerant pollution families. Chlorophyll-a, algal biomass and % algal cover are also used in biological monitoring and can be indicative of change in nutrient level and environmental impact (Paul et al., 2017), with an increase in chlorophyll-a and/or algal biomass often

associated with elevated nutrient levels (Lemley et al., 2016; Omar, 2010; Scheltinga et al., 2004) ,and the presence of indicators species such as diatoms often similarly indicative of impact (Dora et al., 2010).

Tasmanian freshwater hatcheries currently produce over 11 million smolts (juvenile salmon) to supply marine cage production farms around the state. Such production requires a significant input of commercial pellet feed and a resultant output of waste products into the water systems; mainly including soluble nutrients (nitrogen, phosphorus) and suspended solids (uneaten feed pellets, faecal nutrients) that may cause environmental issues (Helfrich and Libey, 1991; Reid, 2007; Turcios and Papenbrock, 2014). The feed conversion ratio within the Tasmanian smolt production is about 1.3:1 in 2003 (Reid, 2007). The review of Wang et al. (2012) states that 1 tonne of fish releases 397 kg C, 50 kg N and 9.3 kg P into the receiving environment. With 11 million smolt at 150 g supply, it is estimated that this would require approximately 2145 tonnes of food and release 978 tonnes of solids, N and P released into the environment (Wang et al., 2012). Tasmanian salmon hatcheries have for many years been employing Recirculating Aquaculture System (RAS) technologies in their production and have been striving to use RAS more and flow to waste systems (such as raceways and outdoor tanks) less. While a few hatcheries in Tasmania have achieved zero waste return to rivers, the hatcheries in this study were at the time of sampling still employing a mix of RAS and flow through strategies. Therefore, total waste production estimates for farms are complex as substantial component of the waste (especially solids) is removed during the RAS process to minimise release to waterways. However, while the interaction between discharges from these hatchery farms and the receiving waters of the rivers has not been published, but there is a general understanding within companies through their in-house monitoring and

subsequent reporting to EPA. In a general perspective release of effluents from hatcheries could have either positive effects such as improved habitat diversity and restoration or negative impacts such as eutrophication (Nobre et al., 2010), deterioration of water quality and adverse changes to macroinvertebrate communities (Pillay, 2008).

To date, monitoring to examine the effects of freshwater salmonid farms on the environment in Tasmanian streams has generally been undertaken by the salmon companies themselves, with results reported to the EPA twice a year. However, due to the concerns regarding water quality outlined above it would seem that an independent assessment of conditions is warranted.

The present study investigates the effects of salmonid aquaculture outfalls on macroinvertebrate assemblages in a number of Tasmania streams, with a particular focus on the different rivers in the Derwent catchment and Northern Tasmania which contains four major salmonid hatcheries. It compares control sites with potentially impacted sites at the outlet of each farm. The degree of impact moving downstream from the outlet will be described in Chapter 4.

3.2 Materials and Methods

3.2.1 Site selection

3.2.1.1 Comparison of farm and non-farm sites in northern Tasmania and the Derwent catchment (Part 1)

Macroinvertebrates were sampled at ten rivers (Figure 3.1, 3.2) in Tasmania namely the St Patricks, Brumbys, Florentine, Russell Falls, Broad, Tyenna End, Styx, Dee, Ouse and Derwent River in autumn 2016 (April 2016). At four of these rivers there were aquaculture farms (St Patricks, Brumbys, Florentine and Russell Falls) and for these rivers there were two sampling

sites; a site upstream of the outfall (hereafter Upstream) and a site sampled at the outlet or the closest accessible site downstream from the outfall (hereafter Downstream). Thus, for this comparison there were 14 sites. Non-farm sites were sampled at locations used by the DEP for water sampling.

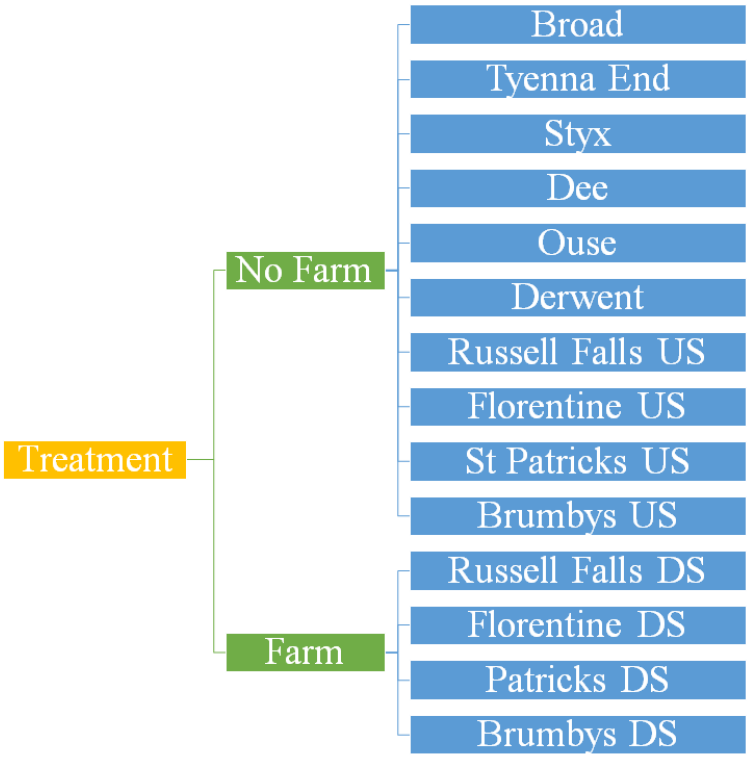


Figure 3.1: The design of sampling of ten streams in Tasmania

Figure 3.2: The map of ten streams sampled in Tasmania (Google map, 2018)
(1: Patricks, 2: Brumbys, 3: Florentine, 4: Broad, 5: Dee, 6: Ouse, 7: Russell Falls, 8: Tyenna End, 9: Styx, 10: Derwent)

3.2.1.2 Comparison of farm (outlet) and non-farm (upstream) sites in the four rivers with four aquaculture farms located (Part 2)

Sampling of macroinvertebrates at the four streams with aquaculture farms: Florentine, Russell Falls, Brumbys Creek and St Patricks River; were done both Upstream, considered as an unimpacted site, and Downstream, considered a potentially impacted site, from the outlet of farms (Figure 3.3, 3.4).

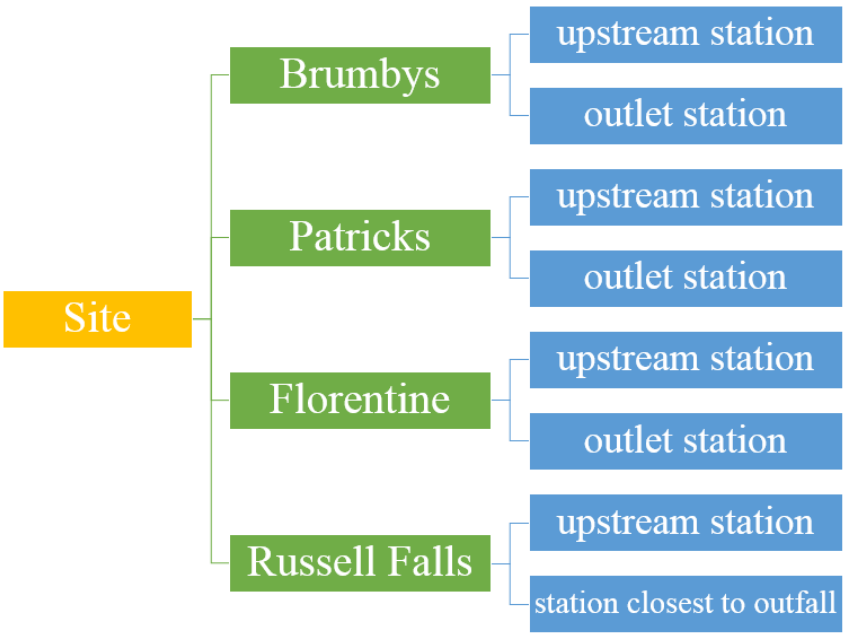


Figure 3.3: The design of sampling of four aquaculture streams in Tasmania

Figure 3.4: The map of upstream and outlet stations at each stream (Google Earth, 2017)

(S1: upstream station, S2: outlet station)

3.2.2 Sampling and processing

This section is described in chapter 2.

3.2.3 Data collection and analysis

3.2.3.1 Data collection:

Data collection is described in chapter 2.

3.2.3.2 Statistical analysis

3.2.1.2.1 Multivariate analyses

Principal coordinates analysis (PCO) was used as a descriptive ordination technique to visualise assemblage differences between treatments, sites and stations. CLUSTER (Clarke and Gorley, 2006a) was also employed to explore the grouping of samples. Data were further explored by similarity percentage analysis (SIMPER) (CLARKE, 1993) if required to test the relative contribution of each taxa to the macroinvertebrate community structure between sites.

Table 3.1: Factor models and the null hypotheses for comparisons

	Comparison	Factors	Null hypothesis
Model 1	Between farm and no farm sites in autumn 2016	Treatment (fixed) with two levels: Farm (4 sites) and No Farm (10 sites) Site (random) nested within treatment	No differences between treatments and sites
Model 2	Between sites and stations upstream and downstream of outlets in autumn 2016	Site (random) with four levels Station (fixed) with two levels: Upstream and Downstream	No differences between sites, stations and no site x station interaction

Permutational multivariate analyses of variance (PERMANOVA) was then used to detect differences in macroinvertebrate assemblages between streams with farms vs. without farms (model 1) and, between stations upstream and downstream of farm outlets at the four streams with farms (model 2, Table 3.1). The PERMANOVA routine in PERMANOVA+ for

Primer 6 (Anderson et al., 2008) is based on any distance matrix, and uses permutation methods to calculate significance values. Data were square root transformed before the Bray Curtis similarities were calculated. The Pseudo-F ratio and P values (α=0.05) were obtained following permutations (N=9999) of the residuals under a reduced mode. Monte Carlo P-values were used instead of permutational *P* values (P_{PERM}) because of low replication. Pair-wise *a posteriori* comparison tests were done to compare each pair of sites and stations.

3.2.1.2.2 Univariate analyses

Biological indices (total abundance, taxa richness and Simpson diversity index) were analysed individually with ANOVA using the same models as multivariate analyses. Data were square root transformed to minimise the impact of dominant values or outliers (Anderson et al., 2008) before the Euclidean distance matrix were calculated which resulted in the same F ratio as in the traditional ANOVA (Anderson et al., 2008). The PERMANOVA routine was used to explore differences in biological indices as the random permutations is less affected by deviations from normality and homogeneity of variances (Anderson et al., 2008). Permutations (N=9,999) were applied to the residuals under a reduced mode. Monte Carlo P-values were used for Pair-wise *a posteriori* comparison tests to compare each pair of treatment, site and station.

3.3 Results

3.3.1 Differences in the macroinvertebrates community between ten rivers including aquaculture and non-aquaculture sites in Tasmania in autumn 2016

3.3.1.1 Assessment of macroinvertebrate assemblages

The multivariate data on community composition showed that there was a significant difference between the communities at the farm sites as compared with those at non-farm

sites. The principal coordinates analysis (PCO) plots clearly distinguishes the Broad (5) and upstream sites of St Patricks (1.1), Russell Falls (3.1) and Florentine (4.1) from the four sites downstream of farming inputs (St Patricks (1.2), Brumbys (2.2), Russell falls (3.2) and Florentine (4.2)) along PCO2 and explains 13.8% of the total variation in the data set. Having said this, 29% of the community variation was captured by PCO1, and it is likely that this reflects the inherent site variability associated with changing river/ habitat conditions. Together the community differences in these two axes account for almost 43% of the overall community variability.

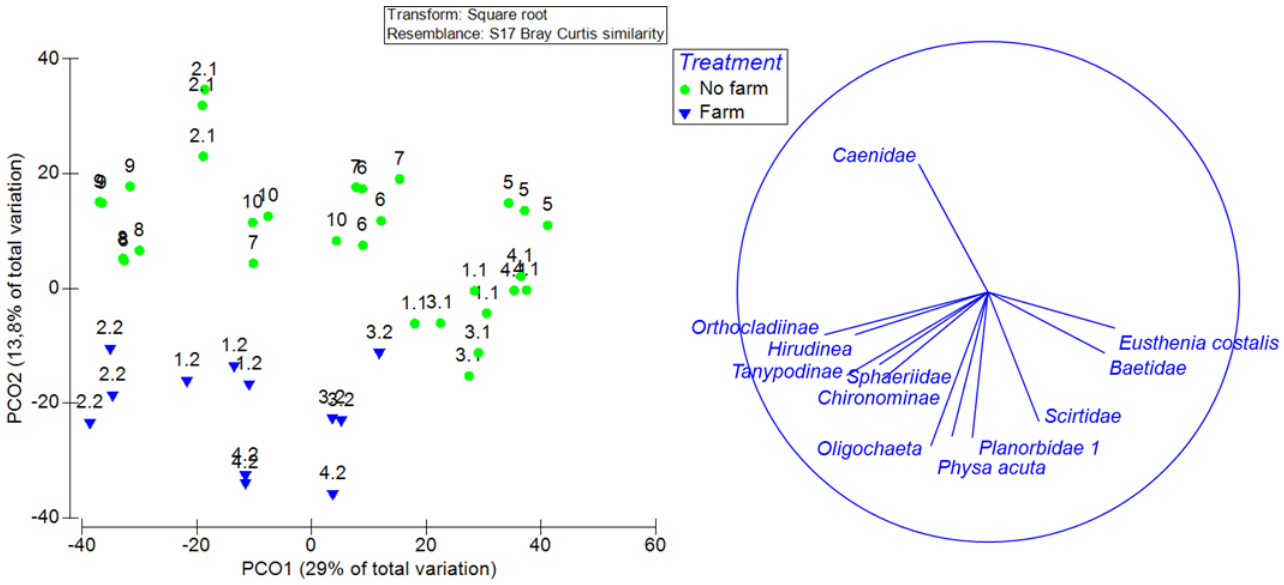


Figure 3.5: Two dimensional PCO plot for macroinvertebrate matrix fauna of 14 sites in Tasmania in autumn 2016. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between sites
(1.1: St Patricks upstream (US), 2.1: Brumbys US, 3.1: Russell Falls US, 4.1: Florentine US, 5: Broad, 6: Tyenna End, 7: Styx, 8: Dee, 9: Ouse, 10: Derwent, 1.2: St Patricks downstream (DS), 2.2: Brumbys DS, 3.2: Russell Falls DS, 4.2: Florentine DS). US: upstream, DS: downstream.

The CLUSTER analysis (Figure 3.6) clearly shows the farm sites grouping together (similarly level of 42%). The cluster analysis also suggests a transition or gradient in the communities at the non-farm sites, with rivers St Patricks (1), Russell Falls (3.1), Florentine (4.1), Broad (5), and Tyenna End (6) being similar in terms of their macroinvertebrate assemblages and

aligning towards the right hand side of PCO 1 (Figure 3.5) and the sites in rivers Brumbys (2.1), Dee (8) and Ouse(9) clustering together towards the left-hand side of PCO1 (Figure 3.5). The Derwent (10) and the Styx (7) communities sit at the interface with replicates aligning with each sample set across the grouping boundary, indicating that at these sites the communities seem to be a transition between the two different river conditions.

The PCO and CLUSTER analysis suggest there were four differentiated groups of sites based on their community assemblages; group 1 (the Broad (5), St Patricks US (1.1), Russell Falls US (3.1) and Florentine US (4.1)), group 2 (the Derwent (10), Brumbys US (2.1), the Dee (8) and the Ouse(9)), group 3 (St Patrick DS (1.2), Brumbys DS (2.2), Russell Falls DS (3.2) and Florentine DS(4.2)), and group 4 (Tyenna End (6) and Styx(7)). Multivariate analysis of variance (PERMANOVA) showed significant differences in macroinvertebrate assemblages between farm and no farm sites ($F_{1,12} = 2.11$, $P_{MC} = 0.046$) indicating that farming has a significant effect on community assemblages and, also between sites within each of those groups ($F_{12,28} = 9.33$, $P_{MC} < 0.001$). Pairwise comparisons of sites within each of the four groupings identified by PCO and CLUSTER showed marked differences in the macroinvertebrate assemblages between all sites in group 1, 2 and 3 (Pairwise PERMANOVA, $P<0.05$) except between sites in group 4 (Tyenna End (6) and Styx (7)).

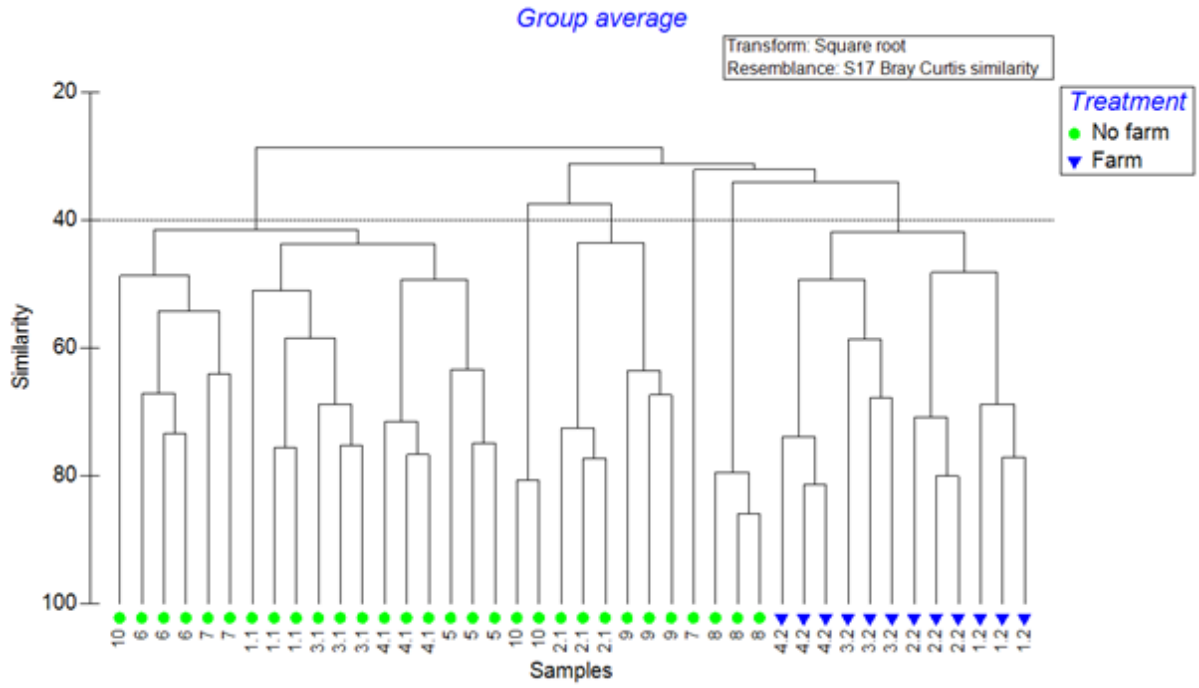


Figure 3.6: Cluster analysis for macroinvertebrate fauna between sites in Tasmania in autumn 2016. Sites labelled as Figure 3.5.

Overlaying the macroinvertebrate data as vector loadings (Figure 3.5) and Simper analyses (Table 3.2, 3.3 & 3.4) shows which species contribute most to the community separation. A number of taxa were associated with farm sites. Scirtidae was associated with communities downstream of the outlet at Russell Falls (farm site, 3.2; Oligochaeta, Planorbidae 1 and *Physa acuta* were associated downstream of the outlet at Florentine (4.2; Orthocladiinae, Sphaeriidae, Tanypodina and Hirudinae were associated with Brumbys (2.2); and Chironomidae and Tanypodinae were associated with downstream of the outlet of St Patricks (1.2) and as such, all these taxa might reasonably be assumed to indicate conditions associated with farming impacts (Figure 3.5). In contrast, Caenidae was positively correlated with PCO2, and were associated with communities upstream of Brumbys (2.1) while *Eusthenia costalis* and Baetidae were associated with Florentine (4.1) and Russell Falls (3.1). This suggests that these taxa are indicators of unimpacted conditions, but also appear able to

differentiate among the different unimpacted sites. Communities at the other end of this gradient (group 3 being characterised more strongly by Oligochaeta, Planorbidae, *Physa acuta,* Hirudina, Orthocladiinae, Chironominae, Tanypodina and Sphaeriidae which are indicative of impacted conditions).

SIMPER analysis shows clear differences in contributions to site communities between the key taxa (Oligochaeta, Planorbidae, *Physa acuta,* Hirudina and Sphaeriidae) that differentiate non-farm and farm as well as upstream and downstream communities. The species differences which are in terms of differences in abundance of same taxa were seen at Group 1 (Broad, St Patricks US, Russell Falls US, Florentine US), Group 2 (Brumbys US, Derwent, Dee, Ouse) and Group 4 (Tyenna End, Styx); which were non-farm sites and key taxa mostly were absent from those sites. In particular, Group 1 has Baetidae being highest; Group 2 and 4 have Hydropsychiadae, Orthocladiinae and Caenidae sometime being highest while Group 3 has Oligochaeta and others being highest. Moreover, the community differences between those three groups and Group 3 (St Patricks DS, Brumbys DS, Russell Falls DS, Florentine DS) were due to different taxa composition, especially the key species. Within Group 3, the species differences were differences in abundance of the same taxa of the community although St Patricks (1.2) and Russell Falls (3.2) were less characterised by Oligochaeta compared to Brumbys (2.2) and Florentine (4.2). In contrast, Oligochaeta were remarkable abundance at Brumbys (2.2) and Florentine (4.2), and the key species played an important role in differentiation of those two sites and other sites as well as their upstream sites

Table 3.2: SIMPER analyses showing the relative taxa contributions (%) to each site of group 1

Rank	Broad (1) (AS = 67.18)	St Patricks US (1.1) (AS = 63.39)	Russell Falls US (3.1) (AS = 70.96)	Florentine US (4.1) (AS = 73.19)
1	Baetidae 25.83%	Baetidae 13.75%	*Atalophlebia australis* 17.25%	Baetidae 22.61%
2	Leptophlebiidae 18.58%	Leptophlebiidae 11.73%	*Lingora sp*. 11.6%	*Costora Delora* 12.02%
3	Hydrobiidae1 12.5%	Scirtidae 10.51%	Baetidae 10.99%	*Eusthenia costalis* 8.11%
4	Leptoceridae2 9.56%	Leptoceridae2 9.62%	Scirtidae 10.9%	Hydropsychidae 6.78%
5	Conoesucidae1 8.5%	Elmidae (L) 7.51%	Elmidae (L) 5.56%	*Lingora sp.* 6.53%
6	Hydropsychidae 8.02%	Hydropsychidae 7.07%	Chironominae 4.98%	Conoesucidae1 6.52%
7	Psephenidae 3.19%	Hydrobiidae1 5.99%	Leptoceridae2 4.36%	Leptophlebiidae 6.17%
8	Philopotamidae 3.07%	Orthocladiinae 4.63%	Orthocladiinae 4.35%	Elmidae (L) 5.02%
9	Elmidae (L) 1.92%	Elmidae (A) 4.28%	Tanypodinae 3.49%	Gripopterygidae 4.75%
10		Simulidae 4.03%	Leptophlebiidae 3.4%	Psephenidae 4.05%
11		*Lingora sp.* 3.88%	Simulidae 3.06%	Hydrobiosidae4 3.25%
12		Hydrobiosidae4 3.71%	Conoesucidae1 2.83%	Elmidae (A) 2.74%

AS: average similarity between three replicates

Table 3.3: SIMPER analyses showing the relative taxa contributions (%) to each site of group 2 and group 4 (AS: average similarity between three replicates)

Rank	Styx (7) (Group 4) (AS = 48.22)	Tyenna End (6) (Group 4) (AS = 69.21)	Dee (8) (Group 2) (AS = 81.60)	Ouse (9) (Group 2) (AS = 64.78)	Derwent (10) (Group 2) (AS = 58.32)	Brumbys US (2.1) (Group 2) (AS = 74.06)
1	Hydropsychidae 13.39%	Hydropsychidae 22.18%	Paramelitidae 22.11%	Orthocladiinae 16.41%	Hydroptilidae 10.09%	Caenidae 17.1%
2	Chironominae 8.78%	Baetidae 16.9%	Simulidae 18.04%	Planorbidae1 9.42%	Orthocladiinae 9.79%	Hydropsychidae 15.13%
3	Simulidae 7.71%	Simulidae 9.24%	Ceinidae 13.94%	Caenidae 6.31%	Hydroptilidae1 9.2%	Orthocladiinae 13.93%
4	Elmidae (L) 5.89%	Orthocladiinae 8.28%	Orthocladiinae 10.93%	Ecnomidae 6.13%	Hydrobiidae1 7.58%	Elmidae (L) 9.26%
5	Oligochaeta 5.71%	Elmidae (L) 6.16%	Chironominae 9.2%	Hirudinea 5.39%	*Costora Delora* 7.3%	Paramelitidae 9.05%
6	Baetidae 5.46%	Tanypodinae 4.88%	Hydropsychidae 7.04%	Chironominae 5%	Caenidae 6.17%	Hydrobiidae1 8.16%
7	Tasimiidae 5.34%	Calocidae 4.74%	Hydrobiidae1 3.85%	Tanypodinae 4.28%	Baetidae 5.37%	Chironominae 6.73%
8	Hydrobiidae1 5.23%	Chironominae 3.85%	Tanypodinae 3.7%	Elmidae (L) 3.71%	Tanypodinae 5.36%	Hydrobiosidae 5.4%
9	Ancylidae 5.12%	Lingora sp. 3.37%	Hydrobiidae2 2.68%	Leptoceridae6 3.63%	Paramelitidae 4.54%	Ceinidae 5.09%
10	Orthocladiinae 4.55%	Ceinidae 3.37%		Hydrobiidae1 3.6%	Elmidae (L) 4.2%	Oligochaeta 5.09%
11	*Atalophlebia australis* 4.34%	Planorbidae2 3.15%		Hydrobiosidae 3.6%	Planorbidae2 3.07%	
12	Planorbidae2 4.05%	Psephenidae 3.14%		*Cura sp.* 3.43%	Calocidae 3.01%	

Table 3.4: SIMPER analyses showing the relative taxa contributions (%) to each site of group 3

Rank	St Patricks DS (1.2) (AS = 71.53)	Brumbys DS (2.2) (AS = 73.92)	Russell Falls DS (3.2) (AS = 61.65)	Florentine DS (4.2) (AS = 76.29)
1	Scirtidae 13.22%	Oligochaeta 23.35%	Baetidae 16.22%	Oligochaeta 27.44%
2	Chironominae 11.62%	Orthocladiinae 11.08%	Hydropsychidae 11.4%	Planorbidae1 15.68%
3	Orthocladiinae 9.67%	Sphaeriidae 8.75%	Chironominae 8.5%	Orthocladiinae 9.86%
4	Elmidae (L) 6.64%	Simulidae 8.02%	*Lingora sp.* 8.03%	*Physa acuta* 8.62%
5	Ceinidae 6.48%	*Physa acuta* 7.33%	Orthocladiinae 6.91%	Chironominae 6.39%
6	Oligochaeta 6.36%	Glossiphoniidae 5.6%	Scirtidae 6.37%	Baetidae 5.64%
7	Paramelitidae 5.85%	Paramelitidae 5.2%	Calocidae 5%	Tanypodinae 3.72%
8	Tanypodinae 5.5%	Tanypodinae 5.17%	Oligochaeta 4.44%	Elmidae (L) 3.66%
9	Simulidae 4.89%	Chironominae 4.83%	Planorbidae1 3.94%	*Costora delora* 2.84%
10	Hirudinea 4.65%	Hydropsychidae 4.57%	Leptophlebiidae 3.79%	Scirtidae 2.23%
11	Hydropsychidae 3.4%	Ceinidae 3.04%	Hydrobiosidae2 3.16%	Psephenidae 2.04%
12	Leptophlebiidae 2.89%	Hirudinea 2.75%	Conoesucidae1 3%	Hydropsychidae 1.97%

AS: average similarity between three replicates

3.3.1.2 SIGNAL 2 index

The overall ratings based on SIGNAL 2 scores were the same between the two methods although including the weighting factor for the most part increased the healthy scores and the unhealthy scores which there was a decrease in site scores but increase in level of pollution (only exception Russell Falls DS). The water quality ratings differed between sites within each group (Table 3.5). Group 1 was the least impacted with all sites rated as *healthy habitat* except St Patricks (1.1) which was rated as *mild pollution.* In group 2, water quality rating was *moderate pollution* for the Dee (8), the Ouse (9) and Brumbys (2.1) while the Derwent (10) was indicated as *mild pollution*. In group 3, St Patrick DS (1.2) and Russell Falls

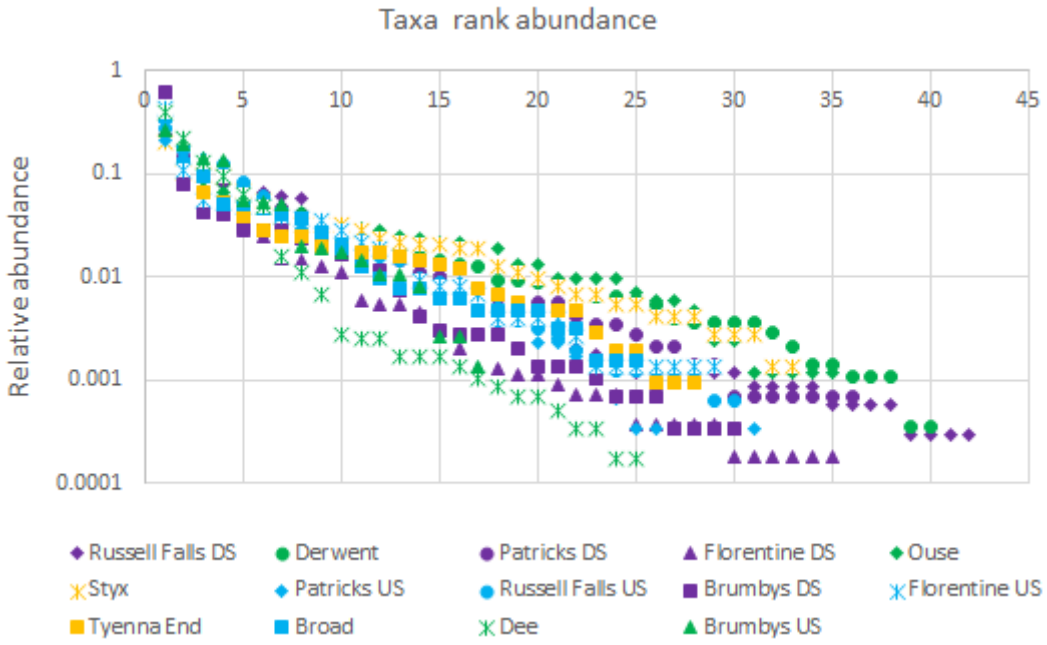
DS (3.2) were rated as *moderate pollution* whilst Florentine DS (4.2) and Brumbys DS (2.2) were rated as *mild* and *severe pollution* respectively. In group 4, water quality rated as *mild pollution* at Tyenna End (6), but *moderate pollution* at Styx (7). Table 3.5 suggests that level of pollution (least to most impacted), respectively, are group 1, group 4, group 2 and group 3.

Table 3.5: Water quality ratings at 14 sites in autumn 2016 based on SIGNAL 2 scores calculated with and without an abundance weighting factor; groupings of sites as per the PCO analysis (US: upstream site, UD: downstream site)

Group	Site	Site score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
1	St Patricks US (1.1)	5.84	mild pollution	6.00	mild pollution
1	Florentine US (4.1)	6.07	healthy habitat	6.61	healthy habitat
1	Russel Falls US (3.1)	6.33	healthy habitat	6.46	healthy habitat
1	Broad (5)	6.36	healthy habitat	6.59	healthy habitat
2	Ouse (9)	4.43	moderate pollution	4.18	moderate pollution
2	Dee (8)	4.84	moderate pollution	4.44	moderate pollution
2	Brumbys US (2.1)	5.00	moderate pollution	4.84	moderate pollution
2	Derwent (10)	5.45	mild pollution	5.28	mild pollution
3	Brumbys DS (2.2)	3.93	severe pollution	3.67	severe pollution
3	St Patricks DS (1.2)	4.72	moderate pollution	4.54	moderate pollution
3	Russel Falls DS (3.2)	4.88	moderate pollution	4.96	moderate pollution
3	Florentine DS (4.2)	5.40	mild pollution	5.33	mild pollution
4	Tyenna End (6)	5.54	mild pollution	5.58	mild pollution
4	Styx (7)	5.55	moderate pollution	5.58	moderate pollution

3.3.1.3 Relative abundance

The dominance-diversity curves indicate some dominant taxa at each site. For the most part the dominance-diversity curves between sites within each group (groups 1-4) were slightly similar with the exception of group 2 (Figure 3.7). Some step dominance-diversity curves at the Brumbys US, the Broad, the Dee and the Tyenna End tended to have a high number of dominant species. Other sites (the Russell Falls DS, the Derwent, St Patricks DS, Brumbys DS and the Ouse) had flatter curves indicating that the communities are more even. Higher relative abundance was seen at sites of group 3, followed by group 2, group 4 and group 1 respectively, although there was variation among sites within these groups (Figure 3.7).



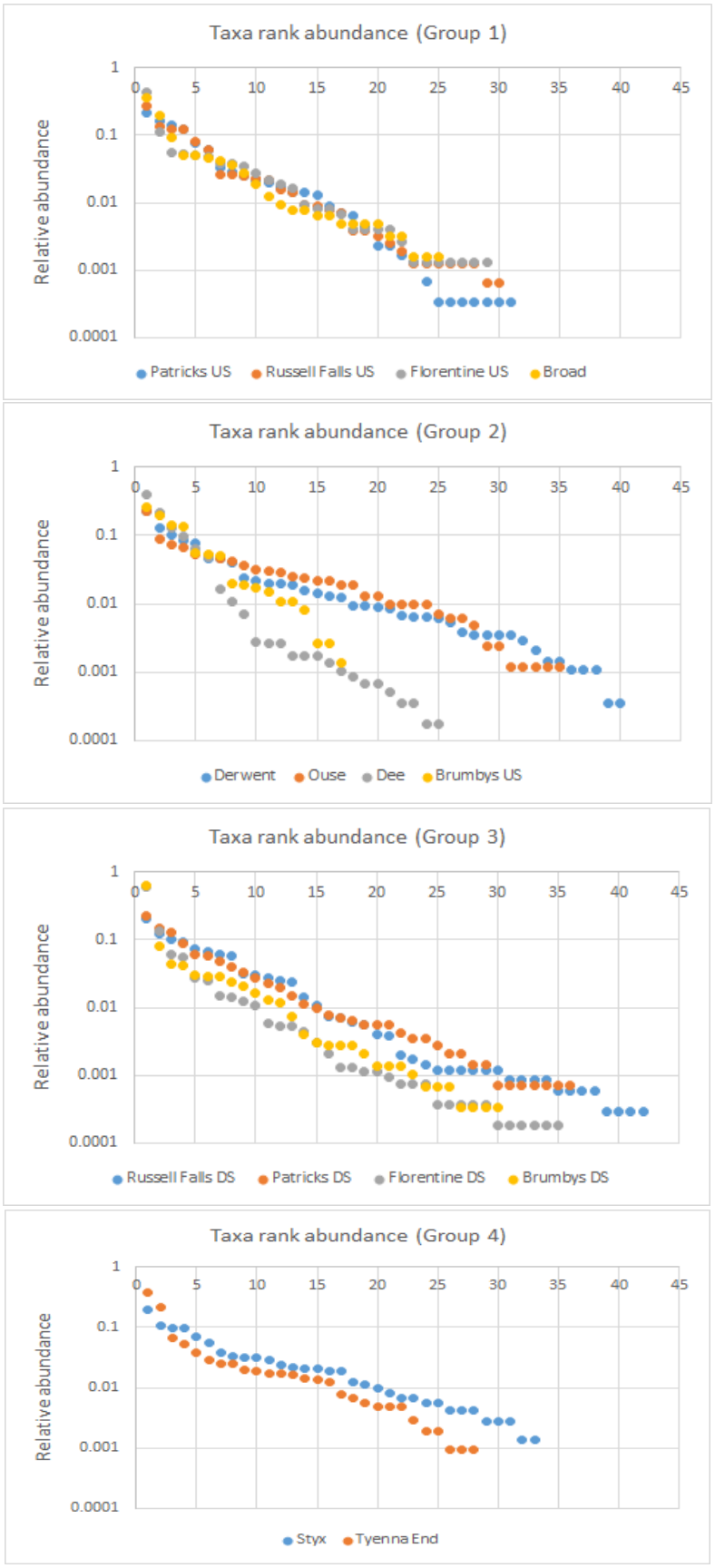


Figure 3.7: The dominance-diversity distribution for 14 sites (top) and each group (1-4) of sites in autumn 2016. Groupings of sites as per the PCO analysis.

For example, the Florentine DS and the Brumbys DS sites had curves which were steep initially and flatter in the latter part; indicating some dominant species.

Russell Falls had highest species diversity (42 taxa), followed by the Derwent (40 taxa), St Patrick DS (36 taxa), the Florentine DS (35 taxa) and the Ouse (35 taxa) respectively (Table 3.6). In contrast, the lowest number of taxa (17 taxa) were found at the Brumbys DS while 25 taxa were recorded at the Broad and the Dee. No sites were without fauna.

The three most dominant taxa across all sites in Group 1 (Broad, St Patricks US, Russell Falls US, Florentine US) were Baetidae, Leptophlebiidae, *Atalophlebia australis,* Scirtidae*, Costora delora* which are all very sensitive to pollution (Table 3.6). In contrast, the most abundant taxa (Oligochaeta, Chironomidae, Planorbidae, *Physa acuta*) in Group 3 (St Patricks DS, Brumbys DS, Russell Falls DS, Florentine DS) are very tolerant to pollution although Baetidae and Scirtidae were respectively high at Russell Falls and St Patricks downstream. Hydroptilidae, Hydrobiidae, Ecnomidae, Hydropsychidae, Paramelitidae and Ceinidae were most abundant taxa at Group 2 (Brumbys US, Dee, Ouse and Derwent) and Group 4 (Styx and Tyenna End) sites. In addition, pollution intolerant species such as Ecnomidae, Planorbidae, Orthocladiinae and Simulidae also inhabited sites in Groups 2, 3 and 4. Based on the tolerance of taxa present, the level of pollution from lowest to highest impacted may be assigned to Groups 1, 4, 2 and 3 respectively.

Table 3.6: The three most dominant taxa and their relative abundance at each of the 14 sites

Site	Total taxa	Total no. individuals	Three most abundant taxa	No. individuals	Relative abundance
Russell Fall DS	42	3445	Baetidae	723	0.21
(Group 3)			Planorbidae 2	414	0.12
			Hydropsychidae	358	0.10
Derwent	40	2823	Hydroptilidae	637	0.23
(Group 2)			Hydrobiidae 1	368	0.13
			Orthocladiinae	281	0.10
St Patricks DS	36	1427	Chironominae	324	0.23
(Group 3)			Scirtidae	211	0.15
			Orthocladiinae	180	0.13
Florentine DS	35	5400	Oligochaeta	3263	0.60
(Group 3)			Planorbidae 1	734	0.14
			Physa acuta	333	0.06
Ouse	35	836	Orthocladiinae	188	0.22
(Group 2)			Ecnomidae	73	0.09
			Planorbidae 1	61	0.07
Styx	33	727	Hydrobiidae 1	145	0.20
(Group 4)			Hydropsychidae	76	0.10
			Simulidae	72	0.10
St Patricks: US	31	3007	Baetidae	633	0.21
(Group 1)			Calamoceratidae	491	0.16
			Leptophlebiidae	429	0.14
Russell Falls: US	30	1580	*Atalophlebia australis*	429	0.27
(Group 1)			Baetidae	209	0.13
			Scirtidae	194	0.12
Brumbys: DS	30	2945	Oligochaeta	1858	0.63
(Group 3)			Orthocladiinae	235	0.08
			Simulidae	127	0.04
Florentine: US	29	750	Baetidae	323	0.43
(Group 3)			*Costora delora*	82	0.11

			Leptophlebiidae	41	0.05
Tyenna End	28	1054	Hydropsychidae	395	0.37
(Group 4)			Baetidae	230	0.22
			Orthocladiinae	69	0.07
Broad	25	633	Baetidae	228	0.36
(Group 1)			Leptophlebiidae	123	0.19
			Hydrobiidae 1	60	0.09
Dee	25	5802	Paramelitidae	2298	0.40
(Group 2)			Simulidae	1262	0.22
			Ceinidae	736	0.13
Brumbys: US	17	745	Caenidae	194	0.26
(Group 2)			Hydropsychidae	147	0.20
			Hydrobiidae 1	106	0.14

3.3.1.4 Total abundance, taxa richness, Simpson diversity index

There were no clear patterns in total abundance, taxa richness and Simpson diversity index between farm and non-farm sites or between the multivariate site groupings identified by PCO and cluster analysis (Figure 3.8). Total abundance, species richness and Simpson diversity of macroinvertebrates did not differ among treatments (farm and no farm sites) but there were however, very large differences between sites within treatments for all three metrics (Table 3.7). Moreover, these metrics often different among sites, reflecting the inherent differences in the different river system although, the patterns for these individual metrics are quite different from those in the full community analysis.

There were no significant differences in taxa richness between the four downstream sites, but there were significant differences in total abundance and Simpson index. Total abundance at St Patricks DS (475 individuals) was significantly lower than that at the Florentine DS (1800 individuals), whereas the Simpson diversity index at both St Patricks DS and Russell Falls DS was significantly higher than that of Brumbys DS and Florentine DS (Figure 3.8).

Table 3.7: ANOVA testing treatment (Tr), Site (Si) and Treatment x Site (TrxSi) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced mode. Pair-wise post hoc comparisons were done for treatment, site and treatment x site. US: upstream, DS: downstream

Source	Df	P_{seudo}-F	P (MC)	Post hoc comparison	P_{seudo}-F	P (MC)	Post hoc comparison
		Total abundance			Taxa richness		
Transformation		Square root			Square root		
Tr	1	2.9427	0.1099		3.5644	0.0896	
Si (Tr)	12	7.742	0.0001		4.5758	0.0005	
				St Patricks DS ≠ Florentine DS			Ouse ≠ St Patricks US, Brumbys US, Broad, Tyenna End, Dee
				Dee ≠ others except for St Patricks US			Derwent ≠ St Patricks US, Brumbys US, Broad, Tyenna End, Dee
				Derwent ≠ others except for St Patricks US			Brumbys US # St Patricks US, Russell Falls US, Tyenna End, Dee, Ouse, Derwent
				Russell Falls US ≠ Florentine US, Broad, Dee, Ouse, Brumbys US, Derwent			
				Broad ≠ Tyenna End			
				Dee ≠ Ouse			
Residuals	28						
		Simpson diversity index					
Transformation		Square root					
Tr	1	0.7849	0.3923				
Si (Tr)	12	5.2353	0.0006				
				Ouse ≠ Brumby US, Florentine US, Broad, Tyenna End, Dee			
				Dee ≠ Derwent, Russell Falls US, St Patricks US			
				St Patricks US ≠ Florentine US			
				St Patricks DS & Russell Falls DS ≠ Brumbys DS, Florentine DS			
Residuals	28						

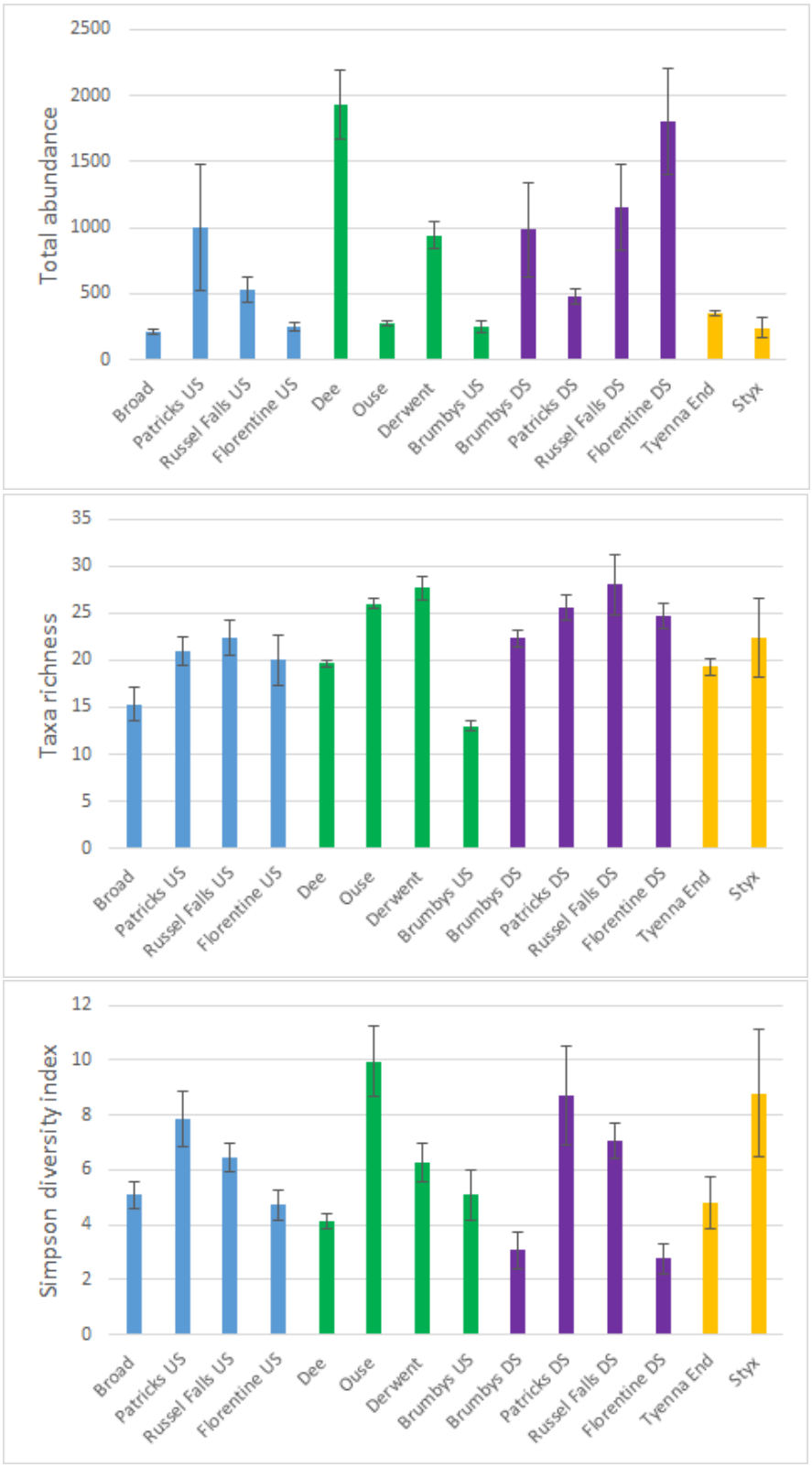


Figure 3.8: Mean (±SE; n=3 replicates) biological indices (Total abundance, Taxa richness, Simpsons diversity index) of macroinvertebrates in 10 sites in the Derwent Catchment and 4 sites in northern Tasmania in autumn 2016. Different colours indicate the site groupings identified by PCO and CLUSTER analyses. Blue = group 1, green = group 2, purple = group 3, yellow = group 4.

The total abundance of the Dee (1934 individuals) was significantly higher than other non-farm sites except for St Patricks US (1002 individuals). Furthermore, total abundance at Russell Falls US (627 individuals) was significantly higher than the Broad (211 individuals), Brumbys US (248 individual), Florentine US (250 individuals), the Ouse (278 individuals) The downstream sites at Brumbys and Florentine had a significantly higher abundance than their upstream sites (Figure 3.8, Table 3.7).

In relation to taxa richness, both the Ouse (26) and the Derwent (28) showed significantly higher levels than St Patricks US (21), Brumby US (13), the Broad (15), Tyenna End (19), the Dee (20) while Brumbys US (13) was significantly lower than St Patricks US (21), Russell Falls US (22), Tyenna End (19) and the Dee (20, Figure 3.8). At farm sites, Russell Falls DS had highest taxa richness, being 28 taxa, followed by St Patricks (approximately 26 taxa), Florentine DS (about 25 taxa) and Brumbys DS (22 taxa). Furthermore, the significant difference in taxa richness between farm and no farm treatment was only seen at Brumbys US and DS. Brumbys DS had a significantly higher taxa richness than Brumby US.

With the Simpson diversity index, the Ouse had the highest Simpson diversity index (9.95), followed by the Styx (8.8), St Patricks US (7.85) and the Derwent (6.25) respectively while the Dee was the site with the lowest diversity index (4.11). The Ouse (9.95) was significantly higher than the Broad (5.1), the Dee (4.12), the Tyenna End (4.82), Brumby US (5.1) and Florentine US (4.72) whilst the Dee (4.12) was significantly lower than the Derwent (6.25), Russell Falls US (6.44) and St Patricks US (7.85). At farming sites, the diversity index of St

Patricks DS (8.71) and Russell Falls DS (7.04) was significantly higher than that of Brumbys DS (3.07) and Florentine DS (2.76).

3.3.2 Differences in the macroinvertebrate community between upstream and downstream stations at four aquaculture sites in Tasmania in autumn 2016

3.3.2.1 Assessment of macroinvertebrate assemblages

The principal coordinates analysis (PCO) clearly separates all of the downstream sites from the upstream sites. Within the upstream sites, Brumbys (1) has a very different community structure compared to all other upstream sites which were similar (Figure 3.9). There appeared to be a gradient of impact among the downstream sites with the communities at site 1 (Brumbys) and 4 (Florentine) being most different to each other. The first and second PCOs axes, which account for 55.9% of the variation, reflecting the effects of both location (sites) and farm (station)(PERMANOVA, $F_{3,16}$= 6.52, P_{MC}= 0.0001). Pair-wise *posteriori* comparisons among stations for each site suggest clear community differences between all sites (pairwise PERMANOVA, P<0.05). The first two axes show the separation of upstream stations from the downstream stations as well as of the Brumbys upstream station from other upstream stations. Moreover, there is little separation between the three upstream stations of the St Patricks, the Florentine and Russell Falls along PCO1 and PCO2.

The four downstream stations are distinguishable along both axes. The Brumbys (2.2) and the Florentine (4.2) are not easily separated along PCO1, but they clearly separate along PCO2. The St Patricks (1.2) did not separate from the Russell Falls (3.2) along PCO2, but there is a clear separation between those two stations along PCO1.

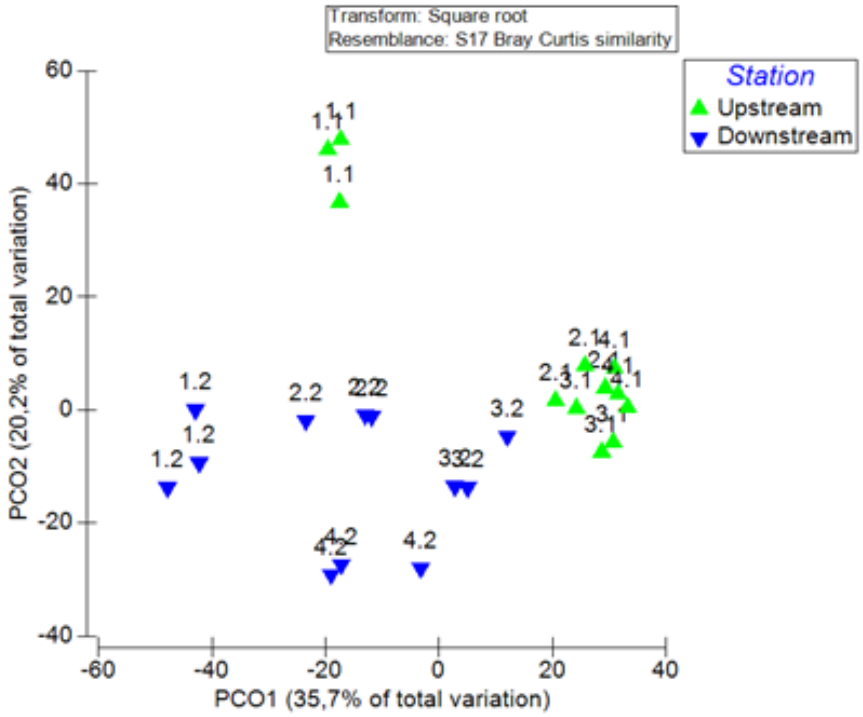


Figure 3.9: The PCO plot for macroinvertebrate fauna Downstream and Upstream from the outlet at four aquaculture sites in autumn 2016. (1: Brumbys, 2: Patricks, 3: Russell Falls, 4: Florentine)

SIMPER analysis supports PCO assessment which shows clear differences between the key taxa differentiating upstream and downstream communities (Table 3.8). Site 2 (St Patricks) & 3 (Russell Falls) were less strongly characterised by key species (Oligochaeta, *Physa acuta*, Planorbidae) with differences between upstream and downstream being more associated with changes in abundance in taxa *per se*. In both cases, Oligochaeta was a key feature. In contrast, at sites 1 (Brumbys) & 4 (Florentine), Oligochaeta played a key role in differentiation of the upstream and downstream community being much more abundant at the downstream sites. Additionally, at both sites 3 and 4, the differentiation between upstream and downstream was also influenced by the presence of taxa in the downstream samples that were largely absent from the upstream (Planorbidae and Oligochaeta at Russell Falls; Planorbidae and *Physa acuta* at Florentine); reflecting a significant community change. Hence in terms of impact, the results suggest level of pollution of site 4 (Florentine) was greater than that of site 1, followed by site 3 (Russell Falls) and site 2 (Patricks).

Table 3.8: SIMPER analyses showing the top five taxa contributions (%) to differences between upstream and downstream stations at each site

(1) Brumbys US vs DS	US	DS		(2) Patricks US vs DS	US	DS	
AD = 67,02	AV.Abundance	AV.Abundance	Contribution %	AD = 61,10	AV.Abundance	AV.Abundance	Contribution %
Oligochaeta	1.91	23.44	21.61	Baetidae	13.49	1.55	9.4
Caenidae	7.71	0	8.09	Leptoceridae	10.06	0.8	7.22
Simulidae	0	6.43	6.76	Leptophlebiidae	11.29	2.24	7.21
Sphaeriidae	0	6.35	6.67	Chironominae	3.56	9.83	5.4
Physa acuta	0	5.32	5.56	Oligochaeta	0	5.98	5.07
(3) Russel Falls US vs DS	US	DS		(4) Florentine US vs DS	US	DS	
AD= 50,34	AV.Abundance	AV.Abundance	Contribution %	AD = 67,28	AV.Abundance	AV.Abundance	Contribution %
Atalophlebia australis	11.81	0	11.02	Oligochaeta	0.8	32.21	25.23
Hydropsychidae	2.36	10.7	8.21	Planorbidae	0	15.62	12.78
Planorbidae	0	8.01	8.06	*Physa acuta*	0	10.23	8.3
Baetidae	8.1	15.28	6.28	Orthocladiinae	1	10.02	7.46
Oligochaeta	0	7.14	6.2	Chironominae	0.33	7	5.53

3.3.2.2 SIGNAL 2 index

The SIGNAL 2 indices indicated similarities in site scores between the two SIGNAL methods (with and without the weighting factor) for all stations at the four sites. Scores at the upstream stations indicated a better water quality rating compared to downstream stations at each corresponding site (Table 3.9). Whilst "healthy habitat" scores were evident at the Russell Falls and Florentine upstream stations, Brumbys and St Patricks had water quality ratings of "moderate pollution" and "mild pollution" respectively for the upstream sites. The sites downstream of the farm inputs appeared markedly deteriorated in all cases with "mild pollution" at the Florentine, "moderate pollution" at St Patricks and Russell Falls, and "severe pollution" at Brumbys (Table 3.9).

Table 3.9: Water quality rating at stations upstream (US) and downstream (DS) of farm outlets at four sites in autumn 2016 based on SIGNAL 2 scores calculated with and without an abundance weighting factor.

Site	Site score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
Brumby US	5.00	moderate pollution	4.84	moderate pollution
Brumby DS	3.93	severe pollution	3.67	severe pollution
St Patricks US	5.84	mild pollution	6.00	mild pollution
St Patricks DS	4.72	moderate pollution	4.54	moderate pollution
Russel Falls US	6.33	healthy habitat	6.46	healthy habitat
Russel Falls DS	4.88	moderate pollution	4.96	moderate pollution
Florentine US	6.07	healthy habitat	6.61	healthy habitat
Florentine DS	5.40	mild pollution	5.35	mild pollution

3.3.2.3 Relative abundance

The dominance-diversity curves differed little between the farm/no farm sites (Figure 3.10). They were relatively flat indicating an even distribution of taxa abundance. There was some evidence for dissimilarities in the dominance-diversity curves of the upstream/downstream stations at the same sites, highlighting differences in relative abundance of upstream and downstream stations. The curves tended to be flatter at upstream stations compared to that of downstream stations, indicating that the upstream stations had higher relative taxa abundance than that of downstream stations.

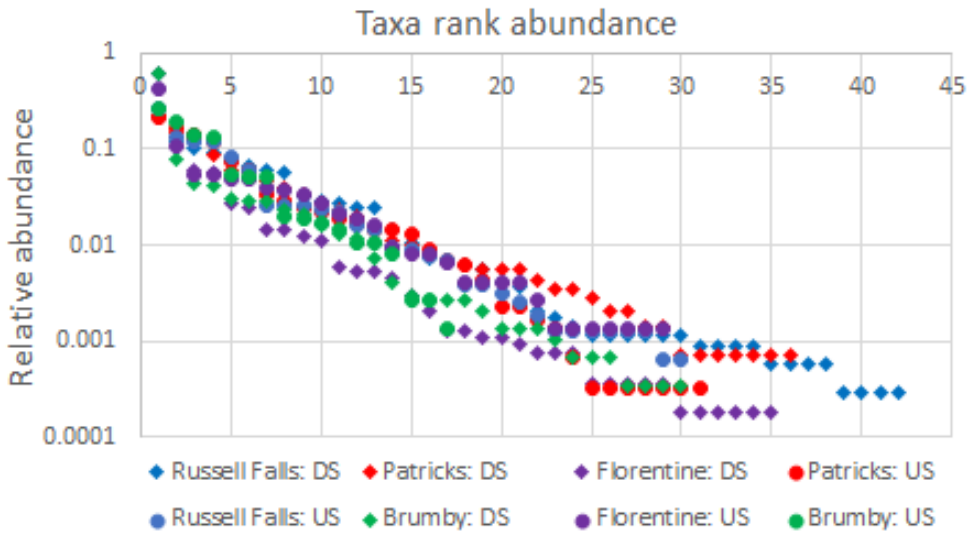


Figure 3.10: The dominance-diversity distribution for stations at four aquaculture streams in autumn 2016 (order of stations in the graph determined randomly by Microsoft Excel)

There were between 17 and 42 taxa across all sites, with consistently higher numbers of taxa at the downstream stations than at the upstream stations. Downstream of Russell Falls recorded the highest number of taxa (42 taxa); followed by the St Patricks DS (36 taxa), the Florentine DS (35 taxa) and the Brumbys DS (30) respectively. The lowest number of taxa was found upstream of the outlet in the Brumbys (17 taxa). The three most dominant taxa and their relative abundance indicated upstream stations were dominated by species typical of

clean water while pollution intolerant species dominated at downstream stations (Table 3.10). Downstream of Florentine and Brumbys were dominated by Oligochaeta suggesting greater impacts compared to St Patrick and Russell Falls (Table 3.10).

Table 3.10: The three most dominant taxa and their relative abundance at eight stations (upstream and downstream) in four sites

	Total taxa	Total no. individuals	Three most abundant taxa	No. individuals	Relative abundance
Brumbys: US	17	745	Caenidae	194	0.26
			Hydropsychidae	147	0.20
			Hydrobiidae 1	106	0.14
Brumbys: DS	30	2945	Oligochaeta	1858	0.63
			Orthocladiinae	235	0.08
			Simulidae	127	0.04
Patricks: US	31	3007	Baetidae	633	0.21
			Calamoceratidae	491	0.16
			Leptophlebiidae	429	0.14
Patricks: DS	36	1427	Chironominae	324	0.23
			Scirtidae	211	0.15
			Orthocladiinae	180	0.13
Russell Falls: US	30	1580	*Atalophlebia australis*	429	0.27
			Baetidae	209	0.13
			Scirtidae	194	0.12
Russell Fall: DS	42	3445	Baetidae	723	0.21
			Planorbidae 2	414	0.12
			Hydropsychidae	358	0.10
Florentine: US	29	750	Baetidae	323	0.43
			Costora delora	82	0.11
			Leptophlebiidae	41	0.05
Florentine: DS	35	5400	Oligochaeta	3263	0.60
			Planorbidae 1	734	0.14
			Physa acuta	333	0.06

3.3.2.4 Total abundance, taxa richness, Simpson diversity index

Total abundance was significantly higher at downstream stations at Brumbys and the Florentine sites but not at Russell Falls (although approximately two times the abundance) and St Patricks (Figure 3.11, Table 3.11). The high abundance at Brumbys and Florentine DS were due to the very high abundance of Oligochaeta (Figure 3.11).

Taxa richness differed between stations and sites (ranging from 13 – 28 taxa) being higher at Brumbys compared to other sites and higher downstream compared upstream (Figure 3.11, Table 3.11). Brumbys upstream has the least taxa (13) while Russell Falls downstream had the highest (28 taxa).

For Simpson diversity index, there were significant differences between sites, but not between stations within each site (Table 3.11). Both Russell Falls and the St Patricks had a higher Simpson diversity than Brumbys and the Florentine (Figure 3.11).

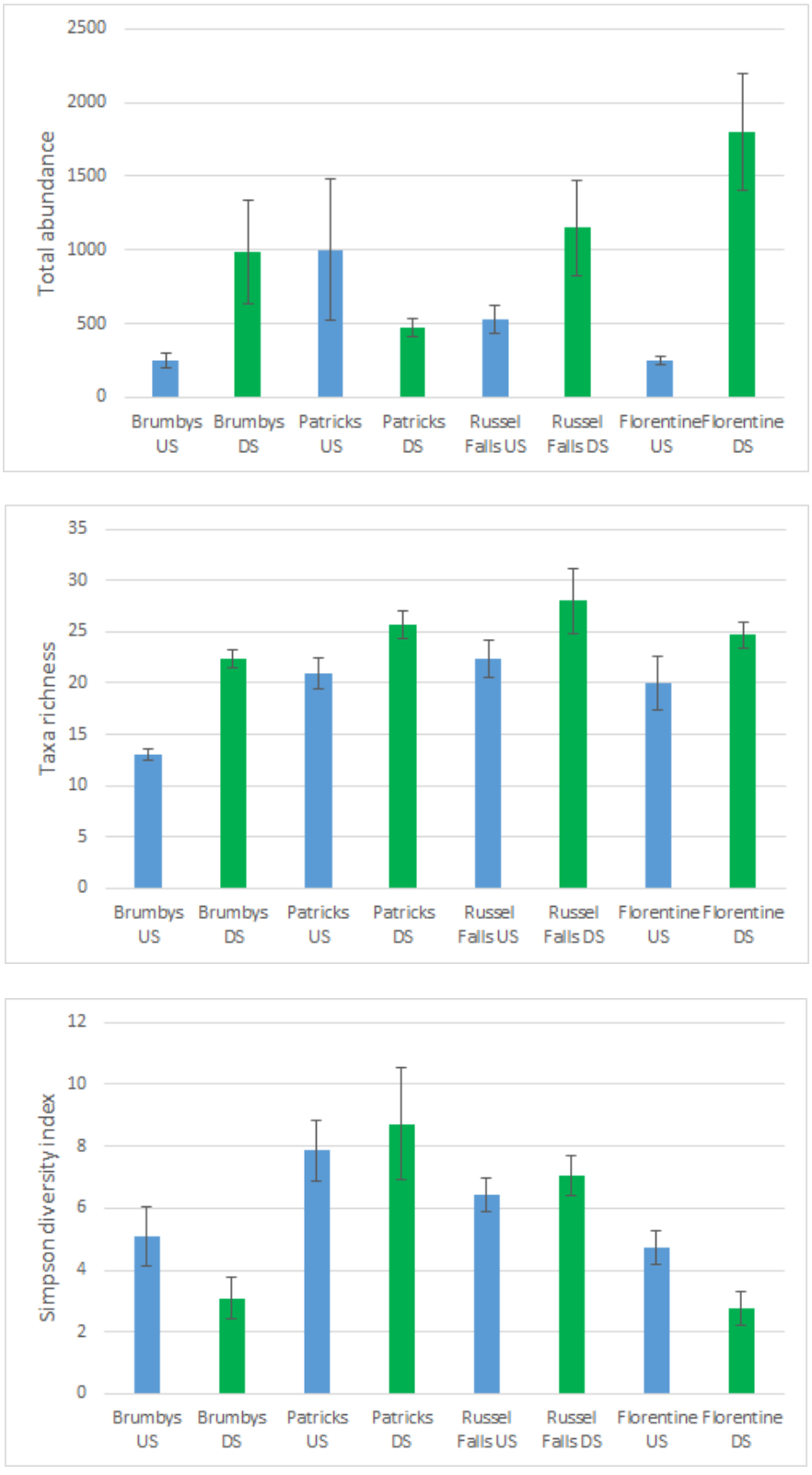


Figure 3.11: Mean (±SE; n=3 replicates) of three biological indices (total abundance, total richness, Simpson diversity index) of macroinvertebrates in eight stations in four aquaculture sites in autumn 2016 (US: upstream, DS: downstream)

Table 3.11: ANOVA testing Site (Si), Station (St) and Site x Station (SixSt) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate communities. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced mode. When significant, pair-wise post hoc comparisons were done for site, station and site x station interaction.

Source	Df	MS	P (MC)	Post hoc comparison	MS	P (MC)	Post hoc comparison
		Total abundance			Taxa richness		
Transformation		Square root			Square root		
Si	3	41.077	0.5214		0.7726	0.0037	Brumbys < others
St	1	698.48	0.2291		2.6813	0.0211	Downstream > Upstream
SixSt	3	306.09	0.0061	Russell Falls US > Florentine US Florentine DS > Patricks DS Brumbys, Florentine: both DS > US	0.1373	0.3285	
Residuals	16	55.944			0.1119		
		Simpson diversity index					
Transformation		Square root					
Si	3	1.282	0.0003	Patricks = Russell Falls > Brumbys = Florentine			
St	1	0.2312	0.3575				
SixSt	3	0.1992	0.1808				
Residuals	16	0.1079					

US: upstream DS: downstream

3.4 Discussion

Previous research has clearly shown that macroinvertebrates vary with stream geomorphology and habitat (Selong et al 1998), as well as biological conditions (impact of aquaculture) (Dumnicka, 2002; Dumnicka and Galas, 2002). In this study, there was also a clear difference in macroinvertebrate community between rivers. Some of these differences presumably reflect the differences in catchment habitat, which can have a marked influence on community structure (Metzeling et al., 2003). A number of studies have shown that local

environmental factors (Mykrä et al., 2007) or catchment features (Richards et al., 1997; Roy et al., 2003a), geophysical factors, land use, as well as anthropogenic impacts (Macedo et al., 2014) can influence community composition. For farm sites, different community structure between farm sites in different regions can be the result of altitude range, terrestrial vegetation, tributary catchment (Brittain et al., 2001), flows (Morgan et al., 1991; Schneider and Petrin, 2017) as well as disturbances at discharge points (Marchetti et al., 2011).

The geomorphology and general habitat of the reference and downstream sites on the same river were similar and therefore it might be reasonable to assume that differences in community structure between those sites would be due to effects of the farm downstream of the outfall. The macroinvertebrate communities at the farm sites were also different from non-aquaculture sites in other rivers as the benthic community can be negatively influenced by organic matter from fish farms (Dumnicka, 2002; Dumnicka and Galas, 2002). Water quality became polluted due to anthropogenic organic factors (Chessman, 1995; Czerniawska-Kusza, 2005) such as land clearing and river regulation (Chessman, 1995). Source of contamination is not only confined to aquaculture however there can be natural and geological factors that might affect macroinvertebrate composition; and agriculture and catchment usage can also influence community composition. The multivariate analysis of the community showed the aquaculture sites quite clearly separated (40% similarity) from other non-aquaculture sites. Within non-aquaculture grouping of sites, the communities tended to reflect the differences resulting from changes in their natural habitat with communities similar habitat and geological conditions grouping together; which was also discussed in chapter 2. The results suggested 4 groupings with group 3 (St Patrick DS, Brumbys DS, Russell Falls DS and Florentine DS) associated with farm impacts and groups 1,2 and 4 associated with the effect of natural

variability. Group 1 (the Broad, Patricks US, Russell Falls US and Florentine US) comprised sites from upland streams, with sandy and rocky substrate, and mostly forestation land use at those sites. Group 2 (the Derwent, Brumbys US, the Dee and the Ouse), and group 4 (Tyenna End and Styx) are located in lowland areas with grazing and agricultural activities. Group 3 sites appeared the most impacted, followed by group 2, and 4 respectively while group 1 was probably cleaner sites. Moreover, within group 3 (farm sites), it was apparent that Brumbys and Florentine had greater impacts from the farm discharge than Patricks and Russell Falls with Oligochaeta, Planorbidae, *Physa acuta,* Sphaeriidae, Hirudinae, and Chironomidae all good indicators of the farm impacts. In contrast, *Eusthenia costalis,* Baetidae and Scirtidae were indicative of cleaner sites

Signal scores without weightings provided very similar ratings to ones with weightings; which is consistent with the previous research (Chapter 2). Therefore, the farms could use the easier score (without abundance) for management. The SIGNAL 2 scores largely support the community categorisation and provided different water quality ratings for various sites. Interestingly, very few of the rivers sampled obtained “good” water quality ratings; suggesting that aquaculture is not the only stressor in these systems. Non-aquaculture sites as in the Derwent, the Tyenna End, the Dee, the Ouse, the Styx all had relatively poor water quality ratings (*mild* or *moderate pollution*). The four rivers with aquaculture farms located on them had better water quality ratings at upstream sites than at their downstream sites. Two of the rivers affected by the farming, the Russell Falls and Florentine, had site scores at sites above the farms which indicated *healthy habitat*, while two then the Patricks and Brumbys were *mildly* and *moderately polluted above the farms*. This indicated water quality of the upstream sites of Patricks and Brumbys themselves might be deteriorated by other sources of human

activities. The water quality ratings of the Florentine and Russell Falls changed from *healthy habitat* above the farms to *mild* and *moderate pollution* respectively at the outlet whilst the Patricks and the Brumbys scored from *mild* and *moderate pollution* at upstream to *moderate* and *severe pollution* at downstream; respectively. The water quality at Brumbys seemed to be more polluted compared to the other three rivers. This may be because this river runs through a lowland agricultural area (degraded system), potentially affected by sheep and cattle manure and other farm nutrient inputs. As a consequence, the waste discharge from the fish farm resulted in relatively heavy pollution at the outlet station in this system and highlights the need to understand background conditions and hence the effect of the farm on top of the background conditions. The research of Armitage et al. (1983a) also found that there were more pollution sensitivity taxa than pollution tolerant taxa in highland rivers and the number of pollution intolerant taxa declined from highland rivers to lowland rivers because of a change in natural habitat. The SIGNAL 2 indicated *healthy habitat* at upstream sites of both Florentine and Russell Falls with a high abundance of *Eusthenia costalis* (stoneflies) and Baetidae (mayflies). The research of Loch et al. (1996b) suggested mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) were indicative of good water quality. Previous studies also affirmed that the abundance of Elmidae (riffle beetles) and Leptophlebiidae (mayfies) decreased substantially from upstream to downstream or were absent at downstream stations (Camargo, 1993) and appear sensitive to pollution (indicated a good water quality stream) (Chessman, 2003a; Gooderham and Tsyrlin, 2002; Goodnight, 1973; MDFRC, 2009). In contrast, the site downstream of Brumbys was deemed to be *severe pollution* and was associated with Orthocladiinae, Sphaeriidae, Tanyppodina and Hirudinae. The site downstream of Patricks was indicated as *moderately polluted* with a high abundance of Chironominae and Tanypodinae. Scirtidae was highly

associated with samples collected downstream of Russell Falls, while Caenidae was abundant at site upstream of Brumbys; where water quality was scored as *moderate pollution*. Oligochaeta, Planorbidae and *Physa acuta* were all associated with the impacted site of Florentine although signal scores *mild pollution*. Aquatic worms (e.g. class Oligochaeta, family Planariidae, genera *Tubifex* and *Limnodrilus*), family Tubificidae, freshwater leeches (class Hirudinea), and larvae and pupae of midges (Chironomidae) are strong indicators of organic pollution (Chessman, 2003a; Gooderham and Tsyrlin, 2002; Goodnight, 1973). Many snails (e.g. *Physa spp.*) are generally present in septic streams while freshwater bivalve molluscs (Sphaeriidae) are indicators of low oxygen condition. Overall, farm sites were characterised by SIGNAL 2 as being more polluted than both their control sites or other non-aquaculture and the key faunal indicators reflected this. This is consistent with previous studies which have shown that a reduction in water quality can result in a concomitant decrease in EPT taxa richness at aquaculture outfalls (Loch et al., 1996b). The finding of Oligochaeta, Planorbidae, *Physa acuta,* Hirudinae and Chironomidae being dominant taxa at the farm affected sites is consistent with other studies which showed higher densities and biomass of Oligochaeta and Chironomidae were associated with polluted water quality (Czerniawska-Kusza, 2005). Czerniawska-Kusza (2005) found that there was a considerable decrease or disappearance in the number of caddisflies (Trichoptera - Limnephilidae, Leptoceridae, Polycentropodidae, Hydropsychidae), mayflies (Ephemeroptera - Heptageniidae, Ephemerellidae, Ephemeridae, Baetidae, Caenidae) at polluted areas; which supported my results as those taxa were abundant at unimpacted sites.

Previously at polluted sites, a reduction in the species richness of macroinvertebrates and abundance of sensitive ‘clean water species’ (Pillay, 2008), and an increase in pollution-

tolerant non-insect taxa has been observed downstream of farms (Selong and Helfrich, 1998). The findings for higher taxa richness at downstream stations in this study contradicts those results and might suggest some enrichment is beneficial. Furthermore, the SIMPER analysis showed that pollution-tolerant taxa made a considerable contribution to the differences in macroinvertebrate communities between upstream and downstream at the same river. Those pollution tolerant taxa were dominant at downstream sites, but less abundant or absent at upstream sites while the number of pollution intolerant taxa decreased from upstream moving downstream. From the farm outlet and downstream, the abundance of specific families such as Chironomidae (midge larvae), Planorbidae (snail), Sphaeriidae (pea shells), Baetidae (mayflies), Tipulidae (tipulids), Empididae (fies) and Simuliidae (black flies) increased significantly (Camargo, 1993) and are tolerant of pollution (Chessman, 2003a; MDFRC, 2009). Loch et al. (1996b) also noted that abundance of pollution-tolerant families; including Chironomidae, Simuliidae, Oligochaeta and Sphaeriidae; was higher just below farm outlet.

Biological metrics (relative abundance, total abundance, taxa richness and Simpson diversity index) did not illustrate the same level of differences in macroinvertebrate communities between sites as each single metric showed different trends. However, those metrics did demonstrate changes in abundance, richness and diversity of macroinvertebrates between farm and non-farm sites as well as the relationship between those indices. Specifically, sites such as Dee, and the downstream sites of Brumbys and Florentine, had a high total abundance but were dominated by certain taxa; resulting in low taxa richness and diversity. In contrast, the remaining sites (Broad, Tyenna End, Styx, Ouse, Derwent, Patricks (upstream and downstream), Russell Falls (upstream and downstream), and upstream sites of Brumbys and

Florentine) had higher taxa richness with fewer dominant taxa resulting in higher diversity. In this research, the Dee and the downstream of Florentine had higher total abundance, and the relative abundance showed that the two dominant taxa (Paramelitidae & Simulidae and Oligochaeta & Planorbidae, respectively) contributed more than 60% of the total abundance at these sites. Similarly, the Derwent, the upstream site at St Patricks, and the downstream sites of the Brumbys and Russell Falls had higher total abundance than that of the Tyenna End, the Styx, the Ouse, the Broad; the upstream sites of Brumbys, Russell Falls and Florentine. The former group of sites had lower diversity indices with the considerably higher abundance associated with enrichment tolerant species which were more prevalent in some river systems than others and also at the downstream (farm affected) sites. The latter group had higher diversity indices as there were a similarity in abundance between taxa in the community at each site. Our results of SIGNAL 2 showed that water quality ratings at the downstream sites were more polluted than at the upstream sites, which was also associated with lower diversity indices. Interestingly, the water quality rating in the downstream Florentine site was only *mild pollution*, however the diversity index was lower than that at upstream site as well as the downstream sites at Brumbys (*severe pollution*), the St Patricks and Russell Falls (*moderate pollution*). This might be because initial build-up of nutrients and waste products may enrich the sediment and increase the habitat diversity (higher taxa richness and diversity index) or it may be the farms are just managing it well. The *severe pollution* scored at Brumbys outlet might be a result of further build-up of wastes which may impact the benthic community. It could also be due to low flows at Brumbys which might cause more solid waste (fine sediment during sampling comprising significant amounts of organic material) to settle to the sediment at downstream sites close to the outlet (Amirkolaie, 2008). This may supply nutrients, resulting in higher taxa richness due to the

higher number of pollution tolerant taxa which diminish water quality rating as well as low diversity index. Armitage (1978) also reported that benthic animals had lower diversity at a station just below an outfall indicating impacts from the farm outfall. In contrast, downstream of the outfall at all four sites there was higher taxa richness as well as higher diversity index at St Patricks and Russell Falls. This exposes in similar results of PCO and dominant; which showed healthier community at St Patricks and Russell Falls downstream than at Brumbys and Florentine downstream. In contrast, Azrina et al. (2006) explained that taxonomic richness and diversity index are higher at better water quality associated with unimpacted or unpolluted condition than at slightly polluted or polluted water quality. Furthermore, Schultheis et al. (1997) found that there was 1.4 – 2.7 time decrease in total abundance and taxonomic richness at the outfall (disturbed station) compared to upstream. The number of macroinvertebrate families are lower at discharged points compared to upstream, but the total number of individuals are highest at the farm effluent and lowest at upstream stations (Camargo, 1993). Low biotic indices at effluent discharge points caused by organic enrichment were also shown by Whitehurst and Lindsey (1990). Pearson and Rosenberg (1978) and Callier et al. (2009) also asserted that a rise in biodeposition due to aquaculture activities caused a reduced macroinvertebrate abundance and richness. Stephens and Farris (2004) showed that minimising negative impacts from effluents on receiving waters resulted in no changes of macroinvertebrate richness as well as of abundance of pollution intolerant taxa such as Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) at outfalls compared with upstream. Our research showed that relative abundance and taxa richness were both consistently higher at the outfall than at upstream in each river on which aquaculture farms located; suggesting that the sites upstream of the outlet are lower in nutrients and the addition of nutrients may be beneficial. This might be because farm impacts

were still at the enrichment level or nutrients were much contained at further downstream than at the outlet; which supplied nutrient for the growth of benthic animals; thus there were an increase in taxa richness at the outlets compare to upstream. The further examination of impacts at further downstream sites from the farm outlets will be explored in chapter 4.

3.5 Conclusion

To conclude, in this study we found

1. that there was evidence of farming effects at the downstream sites but that the level of impact differed partly due to input and system resilience
2. that there were differences in the inherent condition of the rivers that has the potential to affect their ability to assimilate farm inputs (such as some rivers may be more resilient)
3. that whilst simple indices may provide an indication of major effects, they were unable to detect subtle changes and multivariate analyses of community structure were the most useful approach to obtain a clear gradient of effect; which was discussed in chapter 2
4. that SIGNAL 2 matched up with PCO and indicators; and did identify clear indicators of impact and applying SIGNAL 2 and indicators is quick approach to assess and monitor aquaculture farms

Changes in the abundance and community composition of macroinvertebrates among sites were associated with farm waste discharge and natural habitats. The less disturbed sites provide higher abundance of pollution intolerant taxa and macroinvertebrate community composition. Decreased abundance of pollution intolerant taxa and community composition

were found at the sites impacted by the waste discharge. Similarly, stream quality of the less disturbed sites (agricultural, grazing, urban and industrial areas) was cleaner than the impacted sites. The most impacted site was the farm discharge point, possessing a much higher number of pollution tolerant taxa but a lower number of taxa and community composition.

4 Chapter 4: Do stream macroinvertebrate communities differ among stations at different distances from farm outlets and with time?

Abstract

This study examined macroinvertebrate communities upstream from farm outlets, at the farm outlet and downstream of the farm outlet to determine how macroinvertebrate communities recovered moving away from the waste discharge. Stations up to 800 meters downstream of outfalls were sampled at two rivers: Brumbys Creek and Florentine River to investigate the recovery. The less disturbed river (Florentine) had a higher abundance of pollution intolerant taxa and a different macroinvertebrate community structure compared to the more disturbed river (Brumbys) and differences in macroinvertebrate community structure between upstream and downstream stations were observed at both rivers. Very different communities were found at the outlet and the stations immediately downstream of the outlet with a lower abundance of pollution intolerant taxa and a higher abundance of pollution tolerant taxa, indicating those stations were strongly impacted by waste discharge. Similarly, stream quality of the less disturbed stations (upstream and further downstream) was cleaner than the impacted stations (outlet station and closest downstream station). Furthermore, a gradient of recovery in community structure occurred moving downstream at each river; however, downstream communities did not fully recover to those above the outfall within 800 m of the farm outfall. This study has highlighted that stream macroinvertebrate communities are strongly impacted by waste discharge from aquaculture farms and that these impacts can still be observed at least 800 m downstream. Further

research on the recovery processes downstream as well as in recovery over time will help farms monitor and mitigate the effects of farm outputs on receiving water.

4.1 Introduction

Potential impacts of nutrients and solid waste discharge from fish farms on the environment have been well documented (Aure and Stigebrandt, 1990; Bostock et al., 2010; Gowen and Bradbury, 1987; Kelly et al., 1996; Naylor et al., 2000) and have been discussed in the previous chapter (Chapter 3). For freshwater aquaculture, research on macroinvertebrates has mainly focused on the adverse effects of land-based aquaculture farms at the outfall and shows strong effects on stream macroinvertebrate communities (Amirkolaie, 2008; Bostock et al., 2010; Brown; 1996; Brown and King, 1995; Brown, 1998; Carr and Goulder, 1990b; Naylor et al., 2000; Ruiz-Zarzuela et al., 2009). Fewer studies have examined the changes in macroinvertebrate communities downstream of farm outfalls (Brown, 1996; 2001; Camargo, 1992b; 2019; Camargo and Gonzalo, 2007; Guilpart et al., 2012; Selong and Helfrich, 1998; Tello et al., 2010; Webb, 2012b) with limited studies of the distance of recovery from farm effluent.

Studies examining the recovery of downstream macroinvertebrate communities of including Camargo (1992a), Loch et al. (1996a), Selong and Helfrich (1998), Živić et al. (2009) specifically detected the response of macroinvertebrate communities affected by farm effluents. Those studies have demonstrated macroinvertebrate communities did not fully recover within 400 m (Selong and Helfrich, 1998) and 1.5 km downstream (Loch et al., 1996a) from the farm outfalls; but fully recover at 3.5 km downstream from farm outfall (Živić et al., 2009).

In mainland Australia, only one study to date conducted by Webb (2012b) in Victoria, has investigated the impacts of salmonid farms on stream macroinvertebrates. This study examined five trout farms and used multivariate ordination and a Bayesian hierarchical model to examine impacts. The study indicated impacts of individual farms on stream invertebrates although the impacts were not severe; which is similar to our findings in chapter 3 showing different levels of impacts at the four different farms. Webb (2012b) also reported that a higher intensity of production (stream discharge, farm size and annual production) caused greater impacts on stream macroinvertebrates. Nevertheless, the detection of community changes along downstream stretches of the rivers was not recorded.

The results from chapter 3 highlighted there were potential impacts of farm effluents on macroinvertebrate communities at the outfall. Furthermore, it suggested Brumbys Creek and Florentine were more negatively impacted than Patricks and Russell Falls possibly due to different surrounding habitat and the volume of effluent. This study builds on the findings of Chapter 3 to examine the changes in macroinvertebrate communities at varying distances below the outfall. The main objectives of this study were: 1) to examine how the downstream communities respond to the farm effluents by identifying and comparing macroinvertebrate composition at downstream stations with communities upstream and at the farm outlet in two rivers: Brumbys Creek and Florentine; and 2) to investigate whether the community at each station changed over four sampling times, both in relation to season and also following the major flood in 2016.

4.2 Materials and Methods

4.2.1 Site selection

Macroinvertebrates were sampled at the Brumbys Creek and the Florentine River sites where aquaculture farms are located. Brumby's Creek is a small lowland river which receives water from the Great Lake on the central plateau via the hydroelectricity scheme at Poatina and flows through agricultural land (sheep, cattle) and very low density residential areas on farms before joining the Macquarie River. Three weirs are located on Brumby's Creek above the aquaculture farm site, slowing the water flow. In places where I sampled, water plants such as aquatic milfoil and pondweed grow over a silt or mud bottom overlaying rocks and pebbles. Florentine is a highland river with large tributary of the River Derwent that stretches west from near Lake Gordon to the top end of Lake Catagunya. Access is through Sustainable Timber Tasmania land or from the top end of Catagunya. The river starts below Junction Hill at an elevation of 666m and ends at an elevation of 188m flowing into the River Derwent. The Florentine River flows into Lake Catagunya (187m) on its way to joining the River Derwent. Only forestry activities occurred in the Florentine catchment above the references site. The sampling points had a sandy and rocky substrate and were surrounded by forests.

To examine recovery distance from the outfall, macroinvertebrate communities were sampled at the outfall, two stations upstream of the outfall (which acted as control sites): one of these was approximately 10 meters above the outfall with a second station approximately 1000 meters further upstream; and then three (Brumbys) or four (Florentine) stations between 150 – 800m downstream of the outfall (Figures 4.1, 4.2). Stations were selected as randomly as possible depending on access but were consistent in terms of river morphology (e.g. presence of riffle areas). At Brumbys Ck, samples were undertaken in four seasons during

2016: mid-summer (February 2016), mid-autumn (April 2016), mid-winter (July 2016) and mid-spring (October 2016).

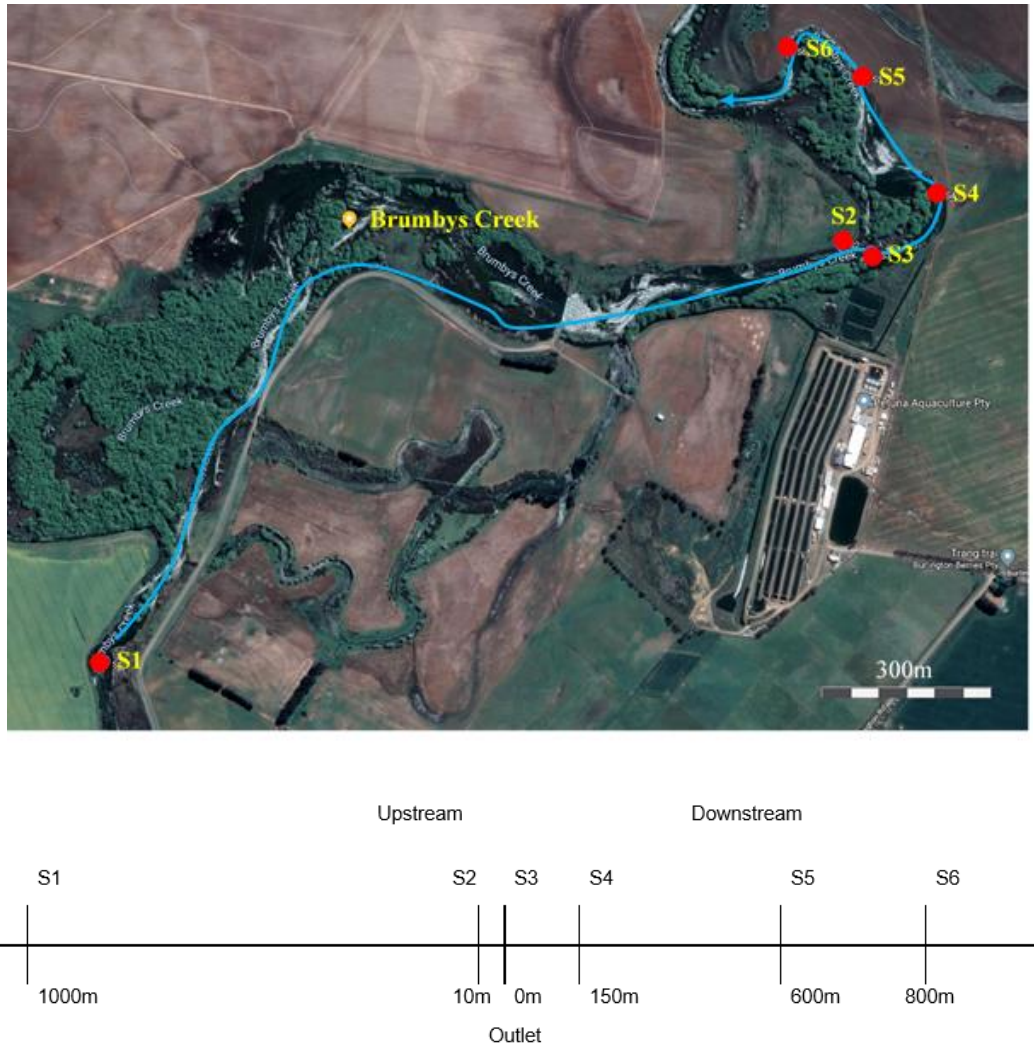


Figure 4.1: General diagram showing the location of sampling stations at Brumbys Ck (S1: upstream 1, S2: upstream 2, S3: outlet, S4: downstream 1, S5: downstream 2, and S6: downstream 3)

At the Florentine site, samples were undertaken in two seasons of summer and autumn in two years (2016 and 2017): summer 2016 (February 2016), autumn 2016 (April 2016), summer 2017 (January 2017) and autumn 2017 (March 2017). Sampling as per Brumbys Creek could not be undertaken as a major flood made the river inaccessible in winter and spring 2016 (Figure 4.3). However, this allowed the impacts of the flood on macroinvertebrates at all stations of the Florentine to be examined.

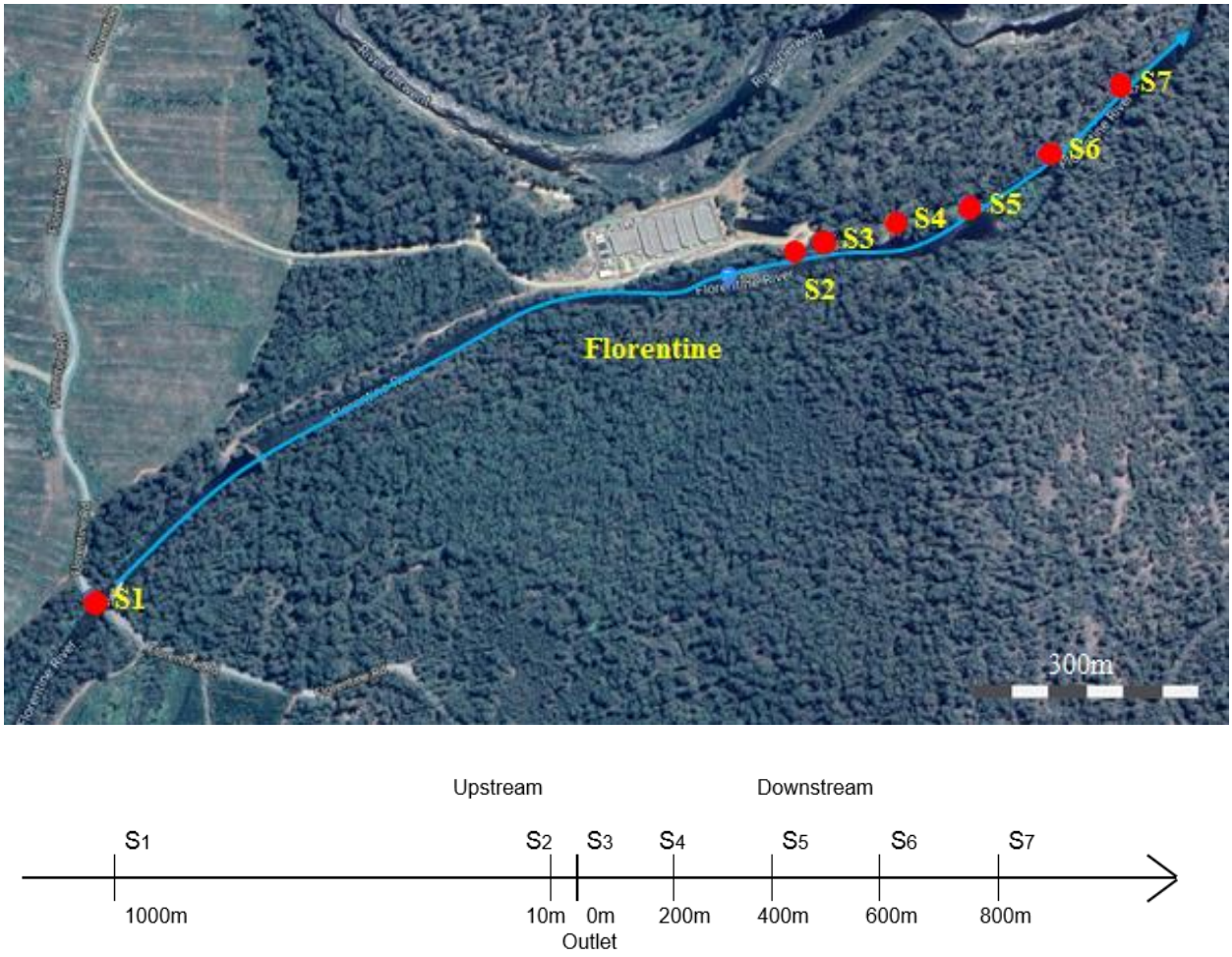


Figure 4.2: General diagram showing the location of sampling stations at Florentine (S1: upstream 1, S2: upstream 2, S3: outlet, S4: downstream 1, S5: downstream 2, S6: downstream 3 and S7: downstream 4)

Furthermore, sites further downstream than about 800m at Florentine and Brumbys were not accessible and did not contain riffle areas to sample.

Figure 4.3: A bridge upstream at the Florentine before (above) and after (below) the flood in winter 2016.

4.2.2 Sampling and processing

Sampling and processing were done as described in chapter 2.

4.2.3 Data collection and analysis

4.2.3.1 Data collection

Data collection as described in chapter 2.

4.2.3.2 Statistical analysis

4.2.3.2.1 Multivariate analyses

Principal coordinates analysis (PCO) was used as a descriptive ordination technique to visualise assemblage differences between sites, stations and seasons. CLUSTER (Clarke and Gorley, 2006a) analyses were then employed to explore the grouping of samples. Data were further explored by similarity percentage analysis (SIMPER) (CLARKE, 1993) to test the relative contribution of each taxa to the macroinvertebrate community between sites.

Table 4.1: Factor models and the null hypotheses for comparisons

	Comparison	Factors	Null hypothesis
Model 1	Differences between stations over four time points	Station (fixed) Season (fixed)	No differences between stations, seasons and station x season interaction
Model 2	Differences between stations in each time point	Station (fixed)	No differences between stations in each single season
Model 3	Differences between time points at each station	Season (fixed)	No differences between seasons at each station

A permutational multivariate analysis of variance (PERMANOVA) was then used to detect differences in macroinvertebrate assemblages between stations as well as between different sampling times. The PERMANOVA routine in PERMANOVA+ for Primer 6 (Anderson et al., 2008) is based on any distance matrix, and uses permutation methods to calculate significance values. Data were square root transformed to balance the contribution of the common and rare taxa before the Bray Curtis similarities were calculated. The PERMANOVAs followed the factorial designs described in Table 4.1. The Pseudo-F ratio and P values (α=0.05)

were obtained following permutations (N=9999) of the residuals under a reduced mode for two factors and unrestricted permutation of raw data for one factor. Monte Carlo P-values were used instead of permutational *P* values (P_{PERM}) because of low replication. Pair-wise *a posteriori* comparison tests were performed to compare each pair of stations and seasons.

4.2.3.2.2 Univariate analyses

Biological indices (total abundance, taxa richness and Simpson diversity index) were analysed individually with ANOVA using the same models as multivariate analyses (Table 4.1). Data were square root transformed to minimise the impact of dominant values or outliers (Anderson et al., 2008) before the Euclidean distance matrix were calculated which resulted in the same F ratio as in the traditional ANOVA (Anderson et al., 2008). The PERMANOVA routine was used to explore differences in biological indices as random permutations are less affected by deviations from normality and homogeneity of variances (Anderson et al., 2008). Permutations (N=9,999) were applied to the residuals under a reduced mode for two factor model and unrestricted permutation of raw data for one factor model. Monte Carlo P-values were used for Pair-wise *a posteriori* comparison tests to compare each pair of stations and seasons.

4.3 Results

4.3.1 Differences in the macroinvertebrate community between six stations in Brumbys Creek over four seasons of 2016

4.3.1.1 Assessment of macroinvertebrate assemblages over four seasons

Principal CO analysis clearly separated macroinvertebrate communities at the outlet station from upstream and downstream stations in all seasons (Figure 4.4). The communities at the two upstream stations could be differentiated; however, the upstream station (2) which was

just above the outfall tended to have similar communities to downstream stations (4 and 5) while the station (1) further upstream had a similar community to the most distant downstream station (6). This indicates recovery may be occurring at the downstream site the greatest distance from the outfall. It would also seem the first and second PCOs (which account for 51.1% of the variation) reflect the effects of both location (stations) and time (season) (PERMANOVA, $F_{15,48}$=5.2, P_{MC}=0.0001). Pair-wise a posteriori comparison among stations over four times suggest clear community differences between almost all stations (pairwise PERMANOVA, P<0.05). The upstream station just above the outfall (2) is distinguishable from the outfall station (3) along PCO1 while the upstream station the greatest distance from the outfall (1) is distinguishable from the outfall station (3) along PCO2. In relation to downstream stations, there is less defined separation between the three downstream stations (4, 5 and 6).

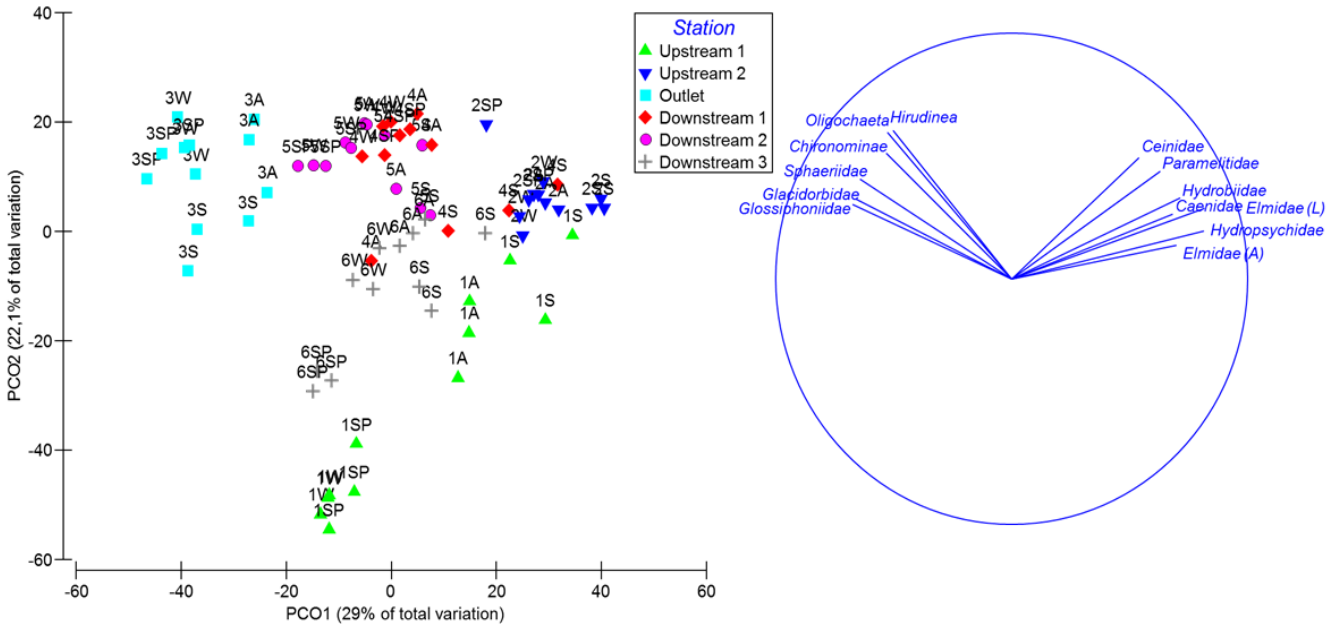


Figure 4.4: PCO plot for macroinvertebrate fauna at six stations at Brumbys Creek over four seasons. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between stations (Numbers on the PCO refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream 1, 5: downstream 2, 6: downstream 3, S: summer. Letters refer to A: autumn, W: winter, SP: spring)

The vector loadings (Figure 4.4) and Simper analysis (Table 4.2) show a correlation between certain macroinvertebrates with certain stations as well as species which contribute significantly to the site separation. Hydrobiidae, Hydropsychidae, Paramelitidae, Caenidae, Elmidae (L), Ceinidae, Elmidae (A) were positively correlated with PCO1, and were associated with upstream 2 (2) while Oligochaeta, Chironominae, Glossiphoniidae, Hirudinea, Sphaeriidae, Glacidorbidae were negatively correlated along PCO1 and positively correlated along PCO2 and were common at the outlet station (3). This suggests that they may be indicative of impacted conditions. Moreover, the three downstream sites (4, 5 and 6) were also characterised by Oligochaeta although the contribution of this key species to the community at those stations was not as high as at the outfall and tended to decline moving downstream (Table 4.2). There is a gradient of impact form right to left in that stations 1 and 2 on the right are reference sites and stations 4,5,6 are intermediate and station 3 on the left is impacted; thus the gradient form right to left indicates increasing impact (separated along PCO1). Furthermore, SIMPER analysis showed there is a high similarity between replicates at each station as well as the outlet station had the most different community composition within the six stations (SIMPER analysis, Table 4.2). There was similar community composition between the upstream station (1 and 2) and downstream stations (4, 5 and 6). The highest similarity in community structure was recorded between the further upstream station (1) and the further downstream station (6); and those two stations were only different in the numbers of each taxa in the communities rather than differences in taxa (Figure 4.4 and Table 4.2). This similarity between stations 1 and 6 indicates that the macroinvertebrate community 800 m downstream of the outfall seemed to recover to be similar level to that upstream. Interestingly, the key taxa (Oligochaeta, Orthocladiinae, and Chironominae) were also present

at the two upstream stations suggesting that the river itself might be impacted by other sources of disturbance.

Table 4.2: SIMPER analyses showing the relative taxa contributions (%) to station (AS: average similarity between three replicates)

Rank	Upstream 1 (1) (AS = 71.48)	Upstream 2 (2) (AS = 78.70)	Outlet (3) (AS = 74.75)	Downstream 1(4) (AS = 72.58)	Downstream 2 (5) (AS = 74.21)	Downstream 3 (6) (AS = 75.95)
1	Orthocladiinae 16.17%	Hydrobiidae 15.83%	Oligochaeta 26.80%	Oligochaeta 22.70%	Oligochaeta 16.19%	Oligochaeta 16.08%
2	Caenidae 12.94%	Hydropsychidae 11.93%	Chironominae 10.59%	Simulidae 9.17%	Chironominae 8.20%	Caenidae 12.20%
3	Chironominae 10.80%	Paramelitidae 11.22%	Orthocladiinae 8.70%	Paramelitidae 8.85%	Caenidae 7.91%	Orthocladiinae 6.92%
4	Hydropsychidae 8.81%	Caenidae 8.82%	Tanypodinae 6.90%	Caenidae 7.07%	Orthocladiinae 6.23%	Tanypodinae 6.84%
5	Paramelitidae 7.47%	Elmidae (L) 6.97%	Glossiphoniidae 6.90%	Hydrobiidae 5.48%	Paramelitidae 5.78	Elmidae (L) 6.70%
6	Elmidae (L) 5.81%	Simulidae 5.73%	Sphaeriidae 5.78%	Elmidae (L) 5.06%	Tanypodinae 5.44%	Chironominae 6.35%
7	Tanypodinae 5.72	Ceinidae 5.73%	Physa acuta 4.26%	Orthocladiinae 4.57%	Hydronbiidae 4.09%	Hydropsychidae 5.59%
8	Simulidae 4.68%	Elmidae (A) 4.58%	Hirudinea 3.98%	Chironominae 4.19%	Elmidae (L) 3.72%	Hydrobiosidae 4.17%
9	Hydrobiidae 4.38%	Orthocladiinae 2.93%	Paramelitidae 3.76%	Hydropsychidae 4%	Ceinidae 3.69%	Oniscigastridae 4.10%
10	Elmidae (A) 4.35%	Phreatoicidae 2.88%	Turbellaria 3.42%	Ceinidae 3.84%	Hydropsychidae 2.95%	Paramelitidae 4.10%
11	Ceinidae 3.56%	Costora Delora 2.66%	Ceinidae 2.95%	Tanypodinae 3.17%	Baetidae 2.73%	Baetidae 3.92%
12	Baetidae 3.18%	Oligochaeta 2.52%	Simulidae 2.70%	Phreatoicidae 3.06%	Leptoceridae4 2.32%	Ecnomidae 3.67%
13	Oligochaeta 2.88%	Lingora sp. 2.40%	Cura sp. 2.54%	Physa acuta 2.13%	Physa acuta 2.28%	Simulidae 2.89%
14		Oniscigastridae 2.31%	Glacidorbidae 2%	Baetidae 1.67%	Phreatoicidae 2.24%	Ceinidae 2.19%
15		Chironomidae 2.03%		Oniscigastridae 1.67%	Hydroptilidae 2.20%	Physa acuta 1.48%

4.3.1.1.1 Assessment of macroinvertebrate assemblages at all stations for each single season

Looking at the six stations within each season, PCO and CLUSTER analysis (Figure 4.5) illustrate that the outlet community (3) was separated from all other stations in summer. Generally, there was a similarity in macroinvertebrate communities (at 60% similarity) between the two upstream stations (1 and 2) and downstream 1 (4) whilst the two further downstream stations (5 and 6) had a similarity level of 60%. Moreover, PCO and CLUSTER (Figure 4.5) illustrates that upstream 1 (1) and downstream stations (4, 5 and 6) had similarities in communities; and that downstream stations 2 (5) and 3 (6) may show recovery from aquaculture impacts. The separation of stations in summer does occur along PCO1 mainly with the clean sites to the left the impacted station to the right and the downstream sites intermediate; also occurs in the autumn PCO.

In autumn, outlet station (3) also separated from the other stations; however, the community tended to be more similar to downstream stations compared to summer (Figure 4.6). There was also a clear separation of the two upstream stations (1 and 2) from the three downstream stations (4, 5 and 6). However, CLUSTER suggests there were 40% similarity in community composition between all stations; suggesting the response of stations is influenced by the stream habitat although, farm effluent appears to cause differences in communities among stations.

In winter, upstream 1 station (1) appears to have a very different community to all of the other stations, showing large separation from all other stations along PCO1 (Figure 4.7). In contrast, there was less defined separation between the three downstream stations (4, 5, and 6). The outlet station (3) and upstream 2 station (2) were distinguishable from downstream

stations along PCO2. PCO and CLUSTER analysis shows that upstream 2 station (2) had a similar community composition to the outlet and downstream stations; indicating farm effluent may influence not only downstream macroinvertebrates but also just upstream from the outlet.

In spring, the upstream 1 station (1) and the further downstream station (6) appeared to have a similar community, but were different from the other stations (2, 3, 4 and 5, Figure 4.8) suggesting the community of the downstream 3 (6) station has partly recovered to be similar to upstream 1 (1). Communities of upstream 2 (2), the outlet (3) and two downstream stations (4 and 5) could all be differentiated with downstream stations (4 and 5) being similar and with the community at the outlet being the most different.

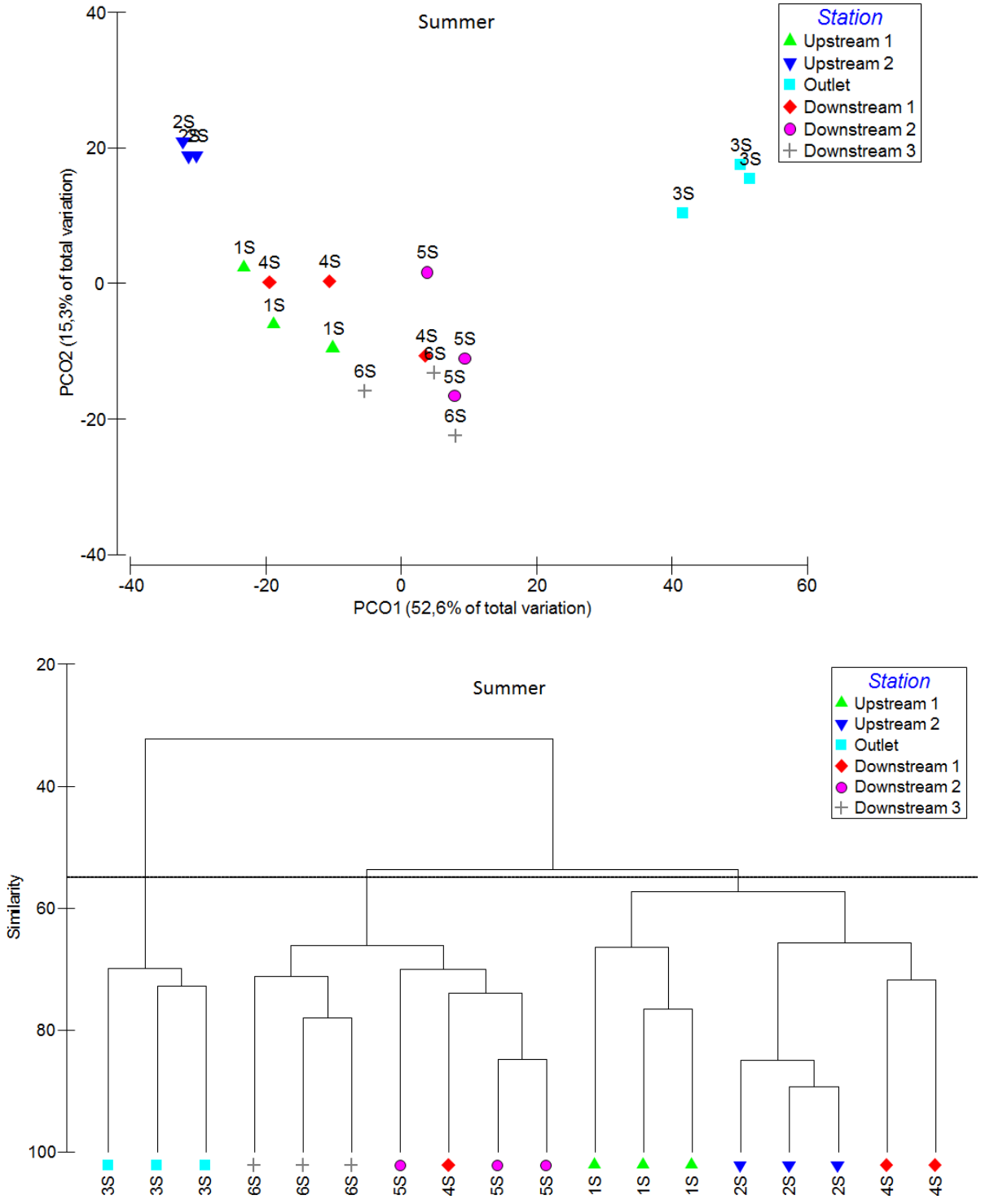


Figure 4.5: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of six stations at the Brumbys Creek in summer 2016 (Numbers refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream 1, 5: downstream 2, 6: downstream 3)

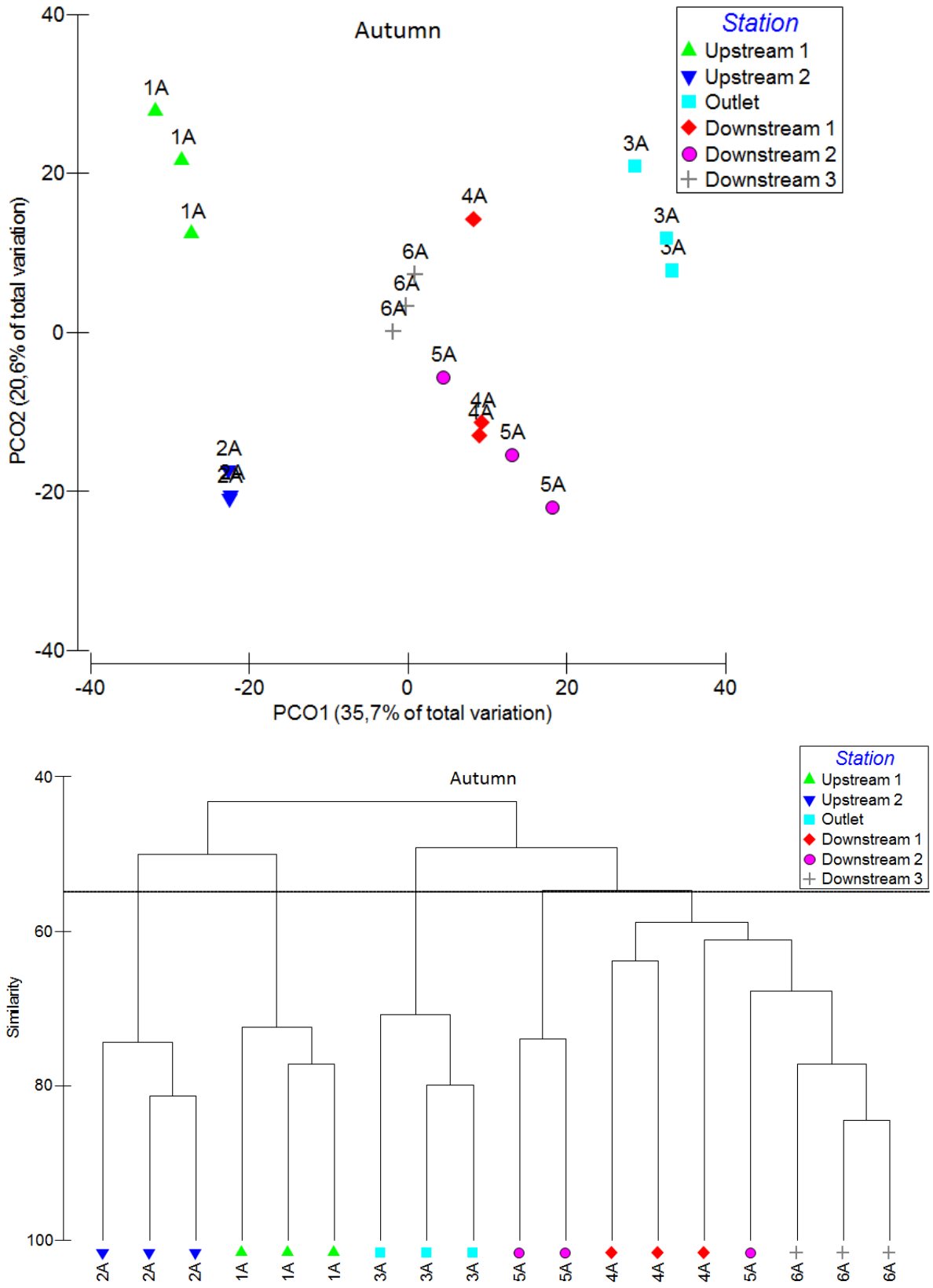


Figure 4.6: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of six stations at the Brumbys Creek in autumn 2016 (Numbers refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream 1, 5: downstream 2, 6: downstream 3)

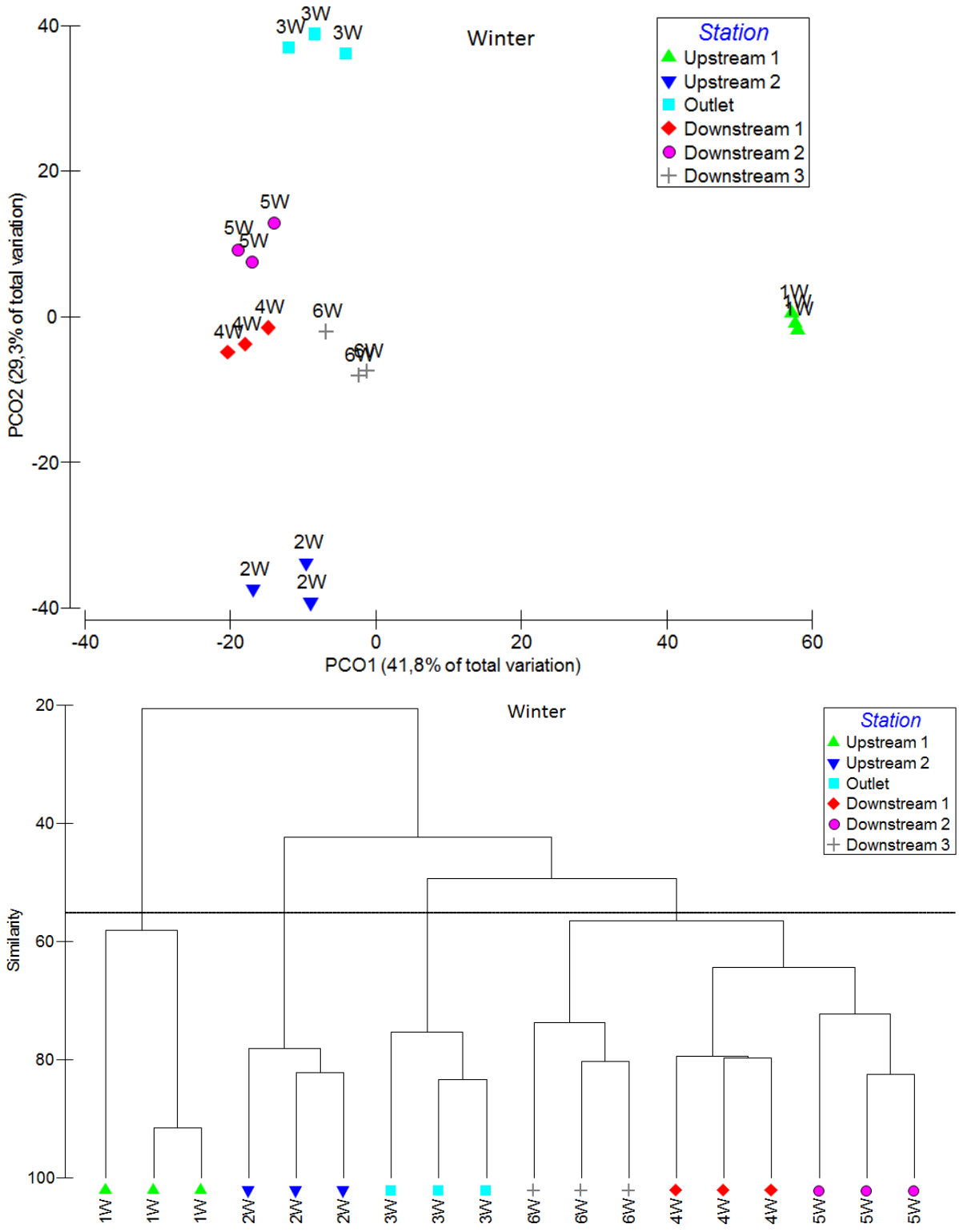


Figure 4.7: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of six stations at the Brumbys Creek in winter 2016 (Numbers refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream 1, 5: downstream 2, 6: downstream 3)

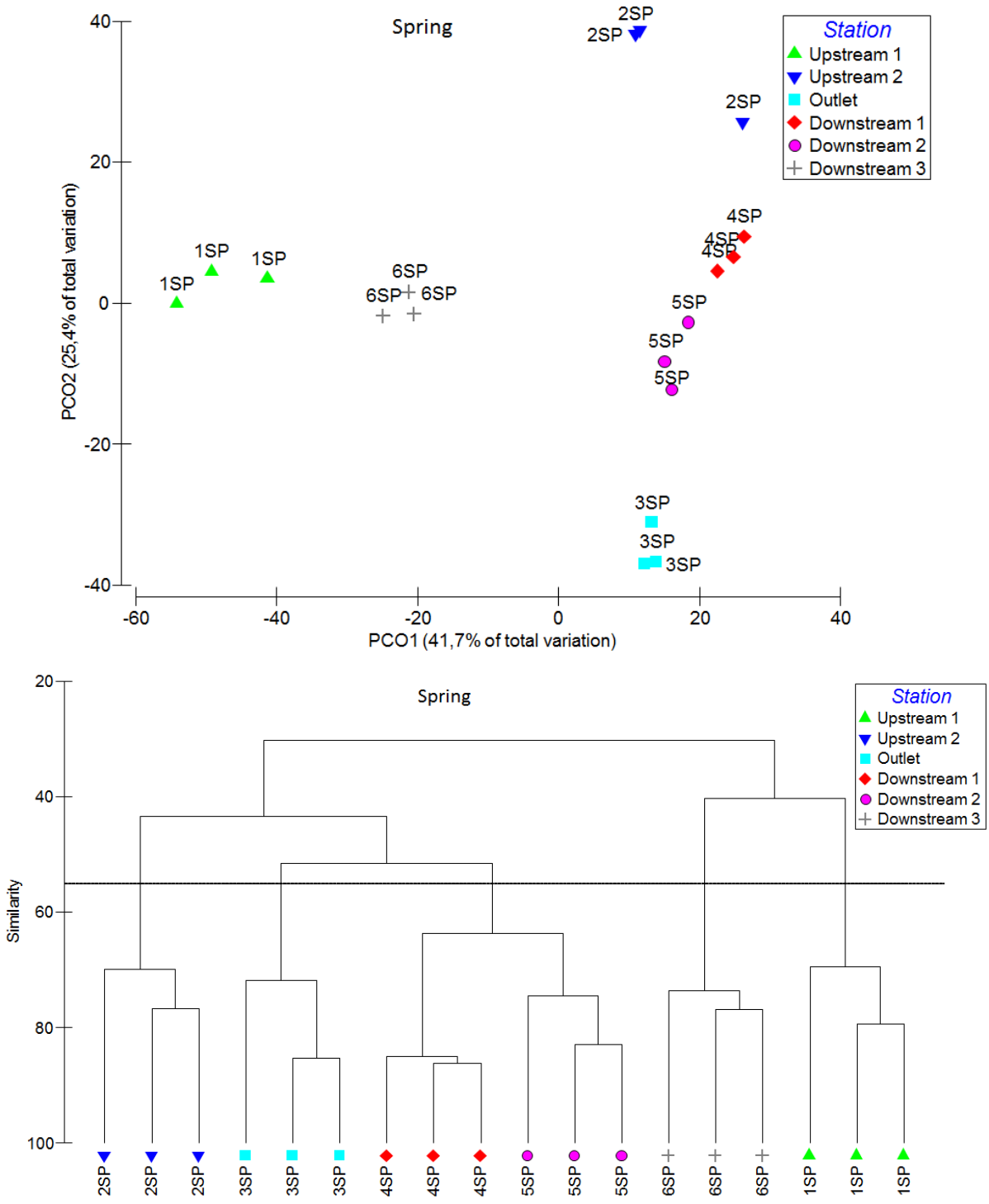
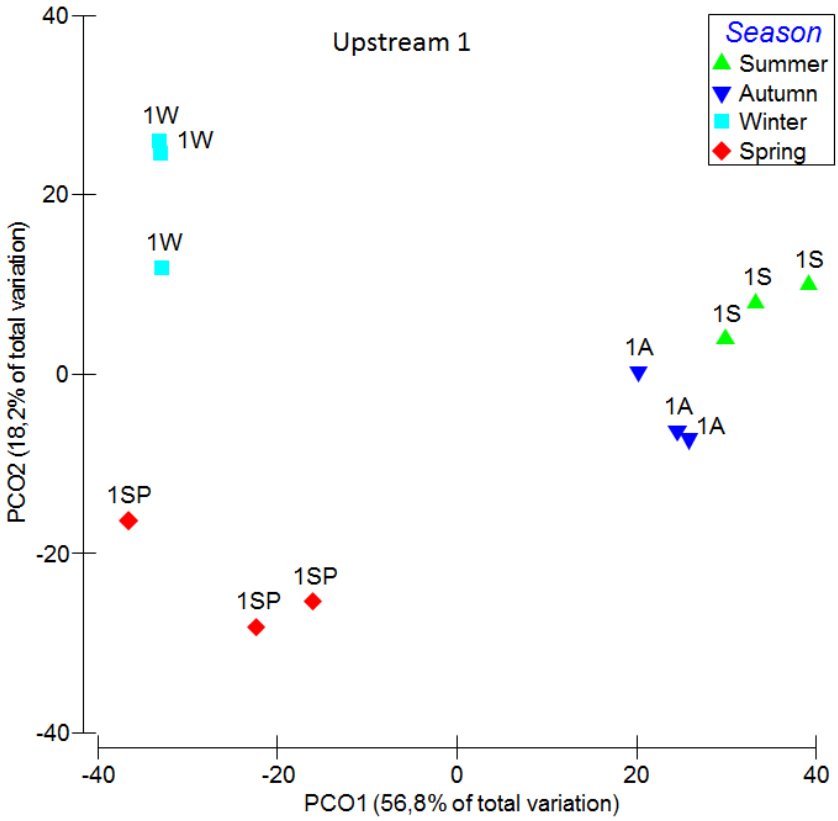
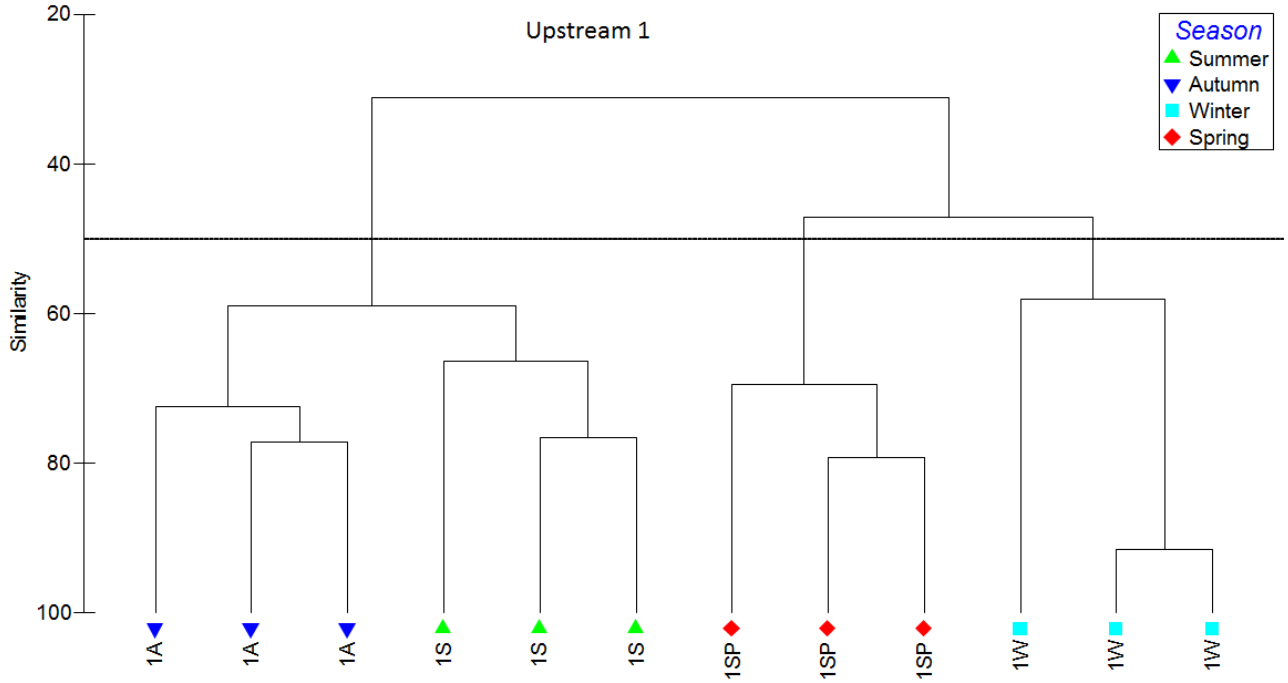


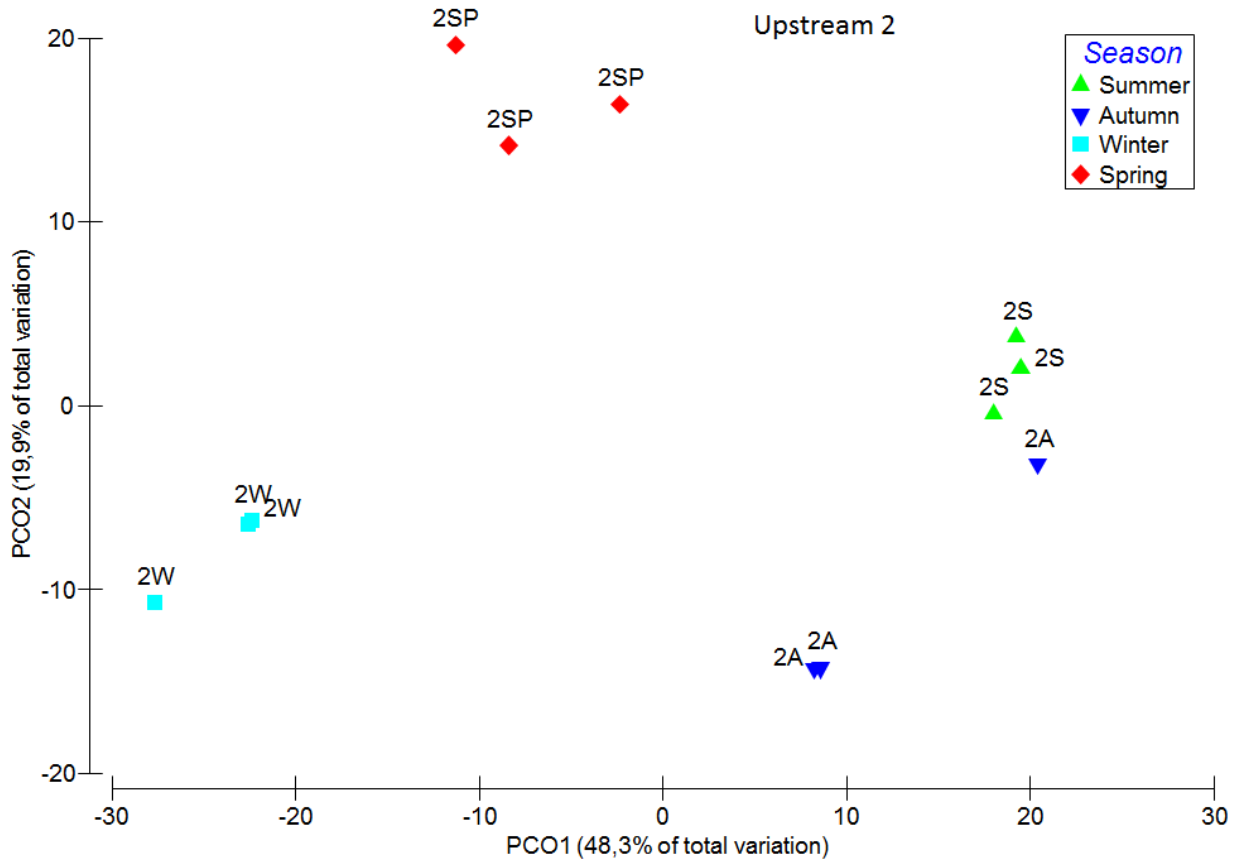
Figure 4.8: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of six stations at the Brumbys Creek in spring 2016 (Numbers refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream 1, 5: downstream 2, 6: downstream 3)

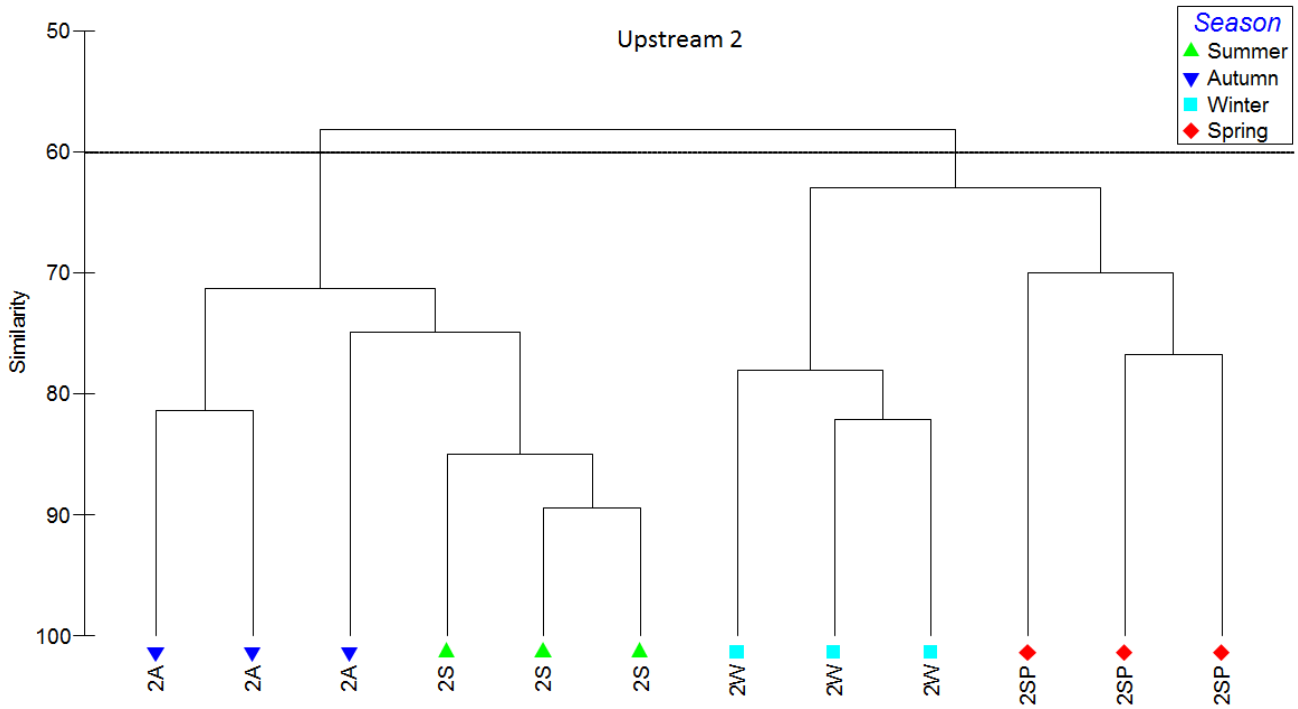
4.3.1.1.2 Assessment of macroinvertebrate assemblages at each station over four seasons

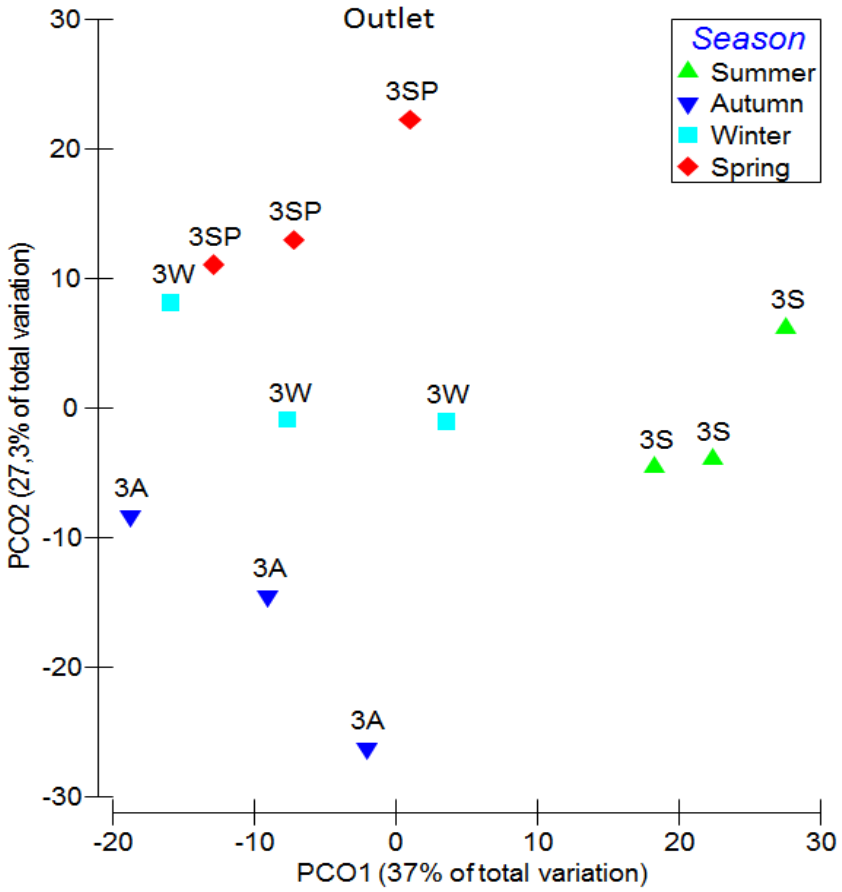
Within each station over four seasons, there was at least 50% similarity in macroinvertebrate community composition between the four seasons at all stations (CLUSTER analysis, Figure 4.9). When comparing seasonal changes within each station, the community composition at the two upstream stations (1 and 2) were more similar in summer and autumn than in winter and spring. There were also similarities in community composition in winter and spring at the outlet (3) and downstream 1 & 2 (4 and 5) while downstream 3 (6) communities were most similar in summer and autumn.

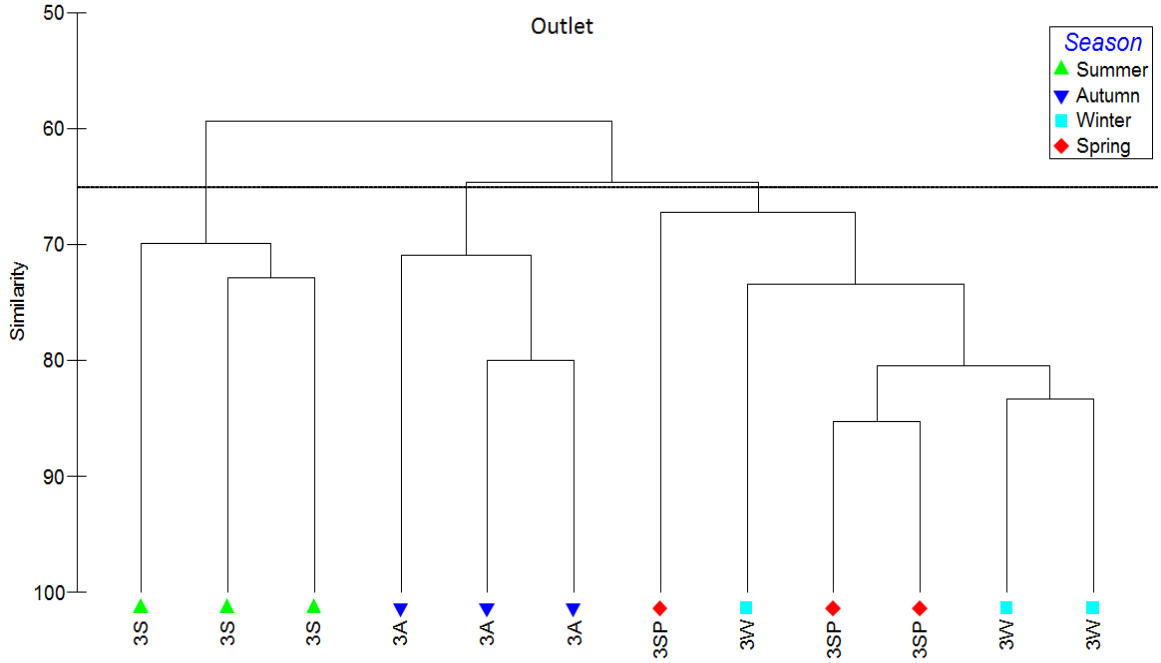

Upstream 1
Season
Summer
Autumn
Winter
Spring
PCO2 (18,2% of total variation)
PCO1 (56,8% of total variation)
40
20
0
-20
-40
1W
1W
1W
1S
1S
1S
1A
1A
1A
1SP
1SP
1SP

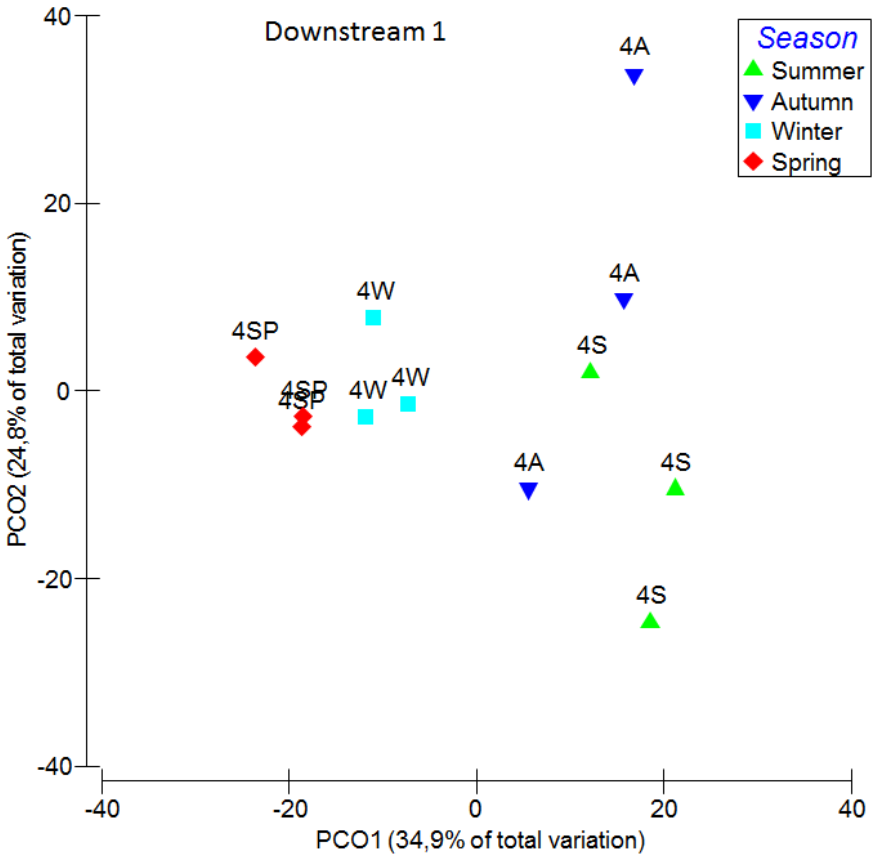

Upstream 1
Season
Summer
Autumn
Winter
Spring
Similarity
20
40
60
80
100
1A
1A
1A
1S
1S
1S
1SP
1SP
1SP
1W
1W
1W

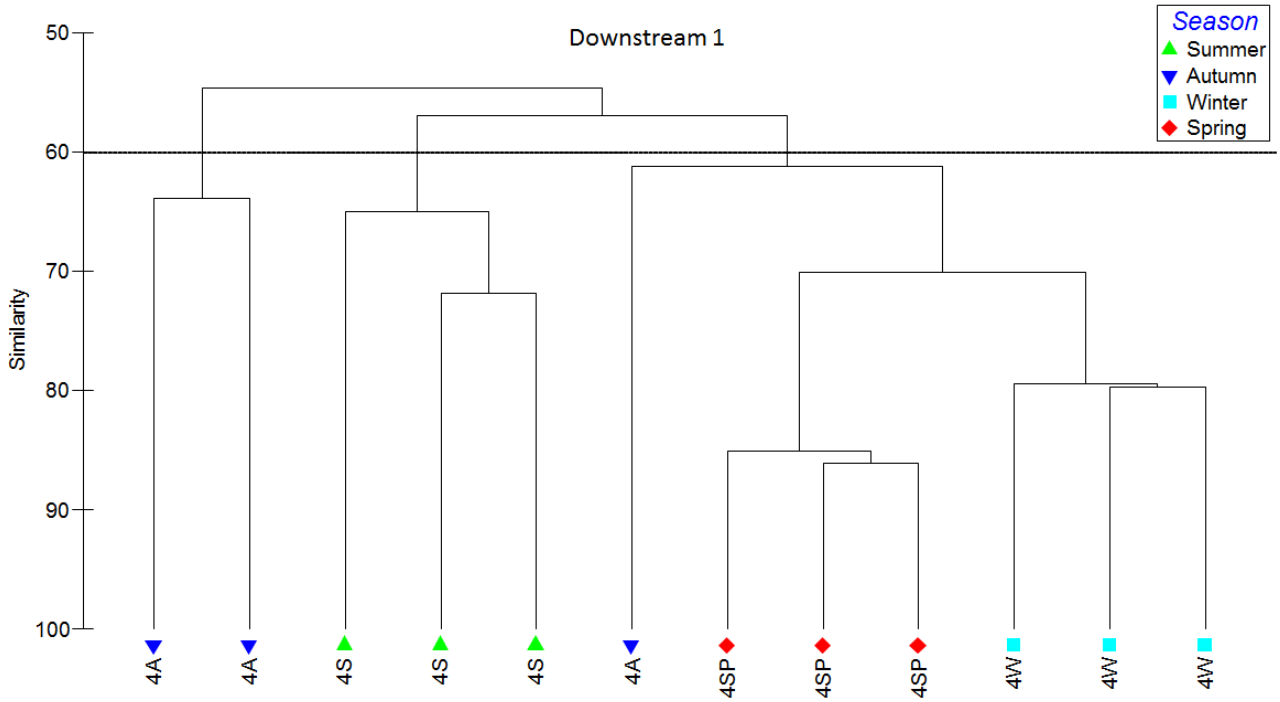

Upstream 2
Season
Summer
Autumn
Winter
Spring
PCO2 (19,9% of total variation)
PCO1 (48,3% of total variation)
2SP
2S
2A
2W
20
10
0
-10
-20
-30
30

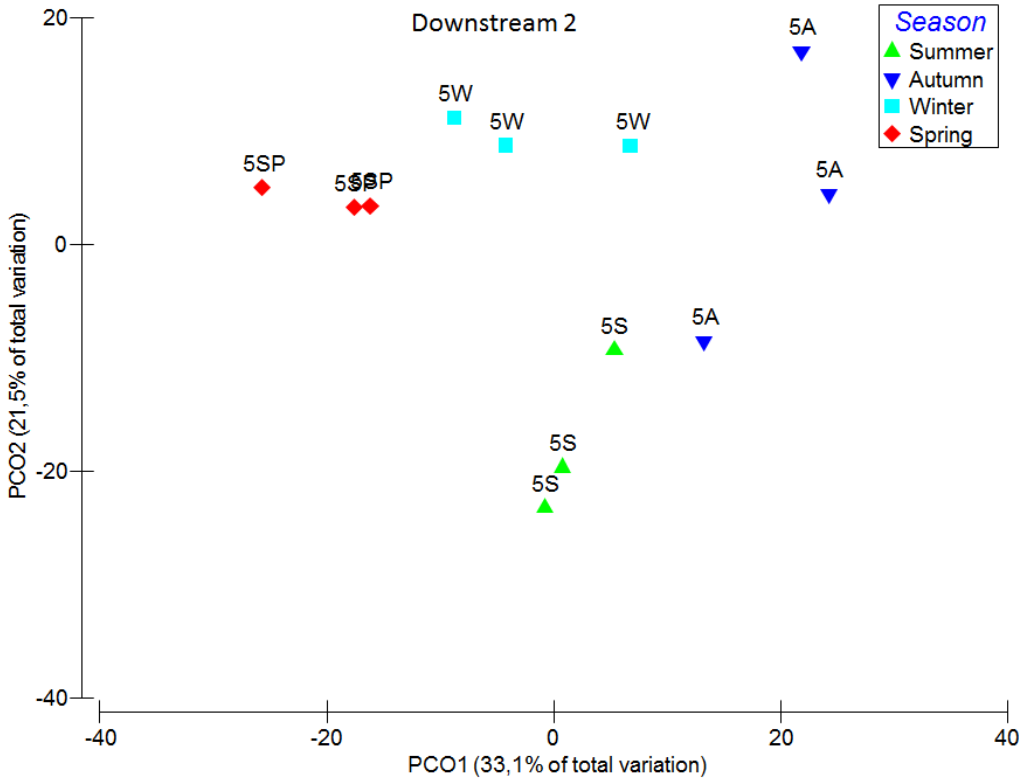

Upstream 2
Season
Summer
Autumn
Winter
Spring
Similarity
50
60
70
80
90
100
2A
2S
2W
2SP

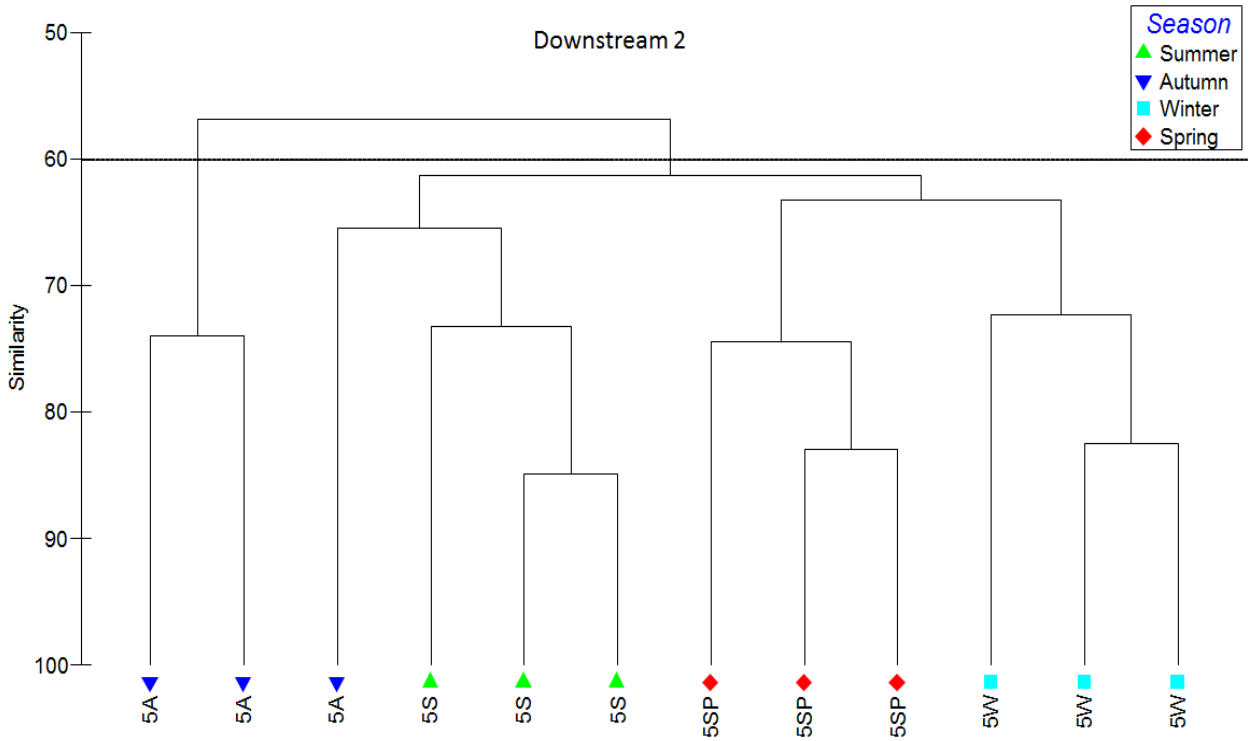

Outlet
Season
Summer
Autumn
Winter
Spring
PCO2 (27,3% of total variation)
PCO1 (37% of total variation)
30
20
10
0
-10
-20
-30
-20
-10
0
10
20
30
3SP
3SP
3SP
3W
3W
3W
3S
3S
3S
3A
3A
3A


Outlet
Season
Summer
Autumn
Winter
Spring
Similarity
50
60
70
80
90
100
3S
3S
3S
3A
3A
3A
3SP
3W
3SP
3SP
3W
3W


Downstream 1
Season
Summer
Autumn
Winter
Spring
PCO2 (24,8% of total variation)
PCO1 (34,9% of total variation)
40
20
0
-20
-40
4A
4W
4SP
4S


Downstream 1
Season
Summer
Autumn
Winter
Spring
Similarity
50
60
70
80
90
100
4A
4S
4SP
4W


Downstream 2
Season
Summer
Autumn
Winter
Spring
PCO2 (21,5% of total variation)
PCO1 (33,1% of total variation)
20
0
-20
-40
-40
-20
0
20
40
5A
5W
5SP
5S


Downstream 2
Season
Summer
Autumn
Winter
Spring
Similarity
50
60
70
80
90
100
5A
5S
5SP
5W

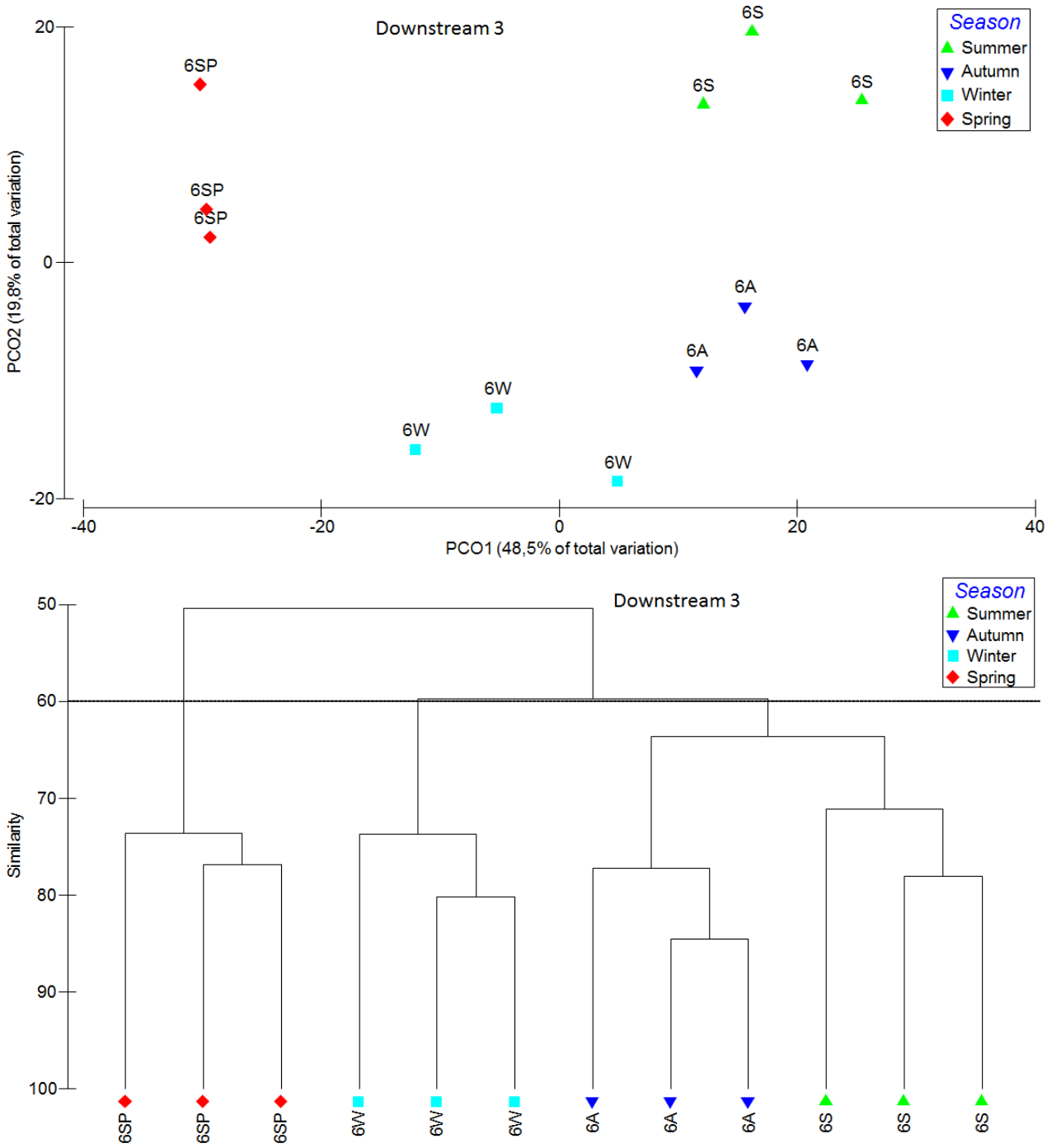


Figure 4.9: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of each station (1 – 6) at the Brumbys Creek (Numbers refer to 1: upstream 1, 2: upstream 2, 3: outlet, 4: downstream1, 5: downstream 2, 6: downstream 3) across seasons (Letters refer to S: summer, A: autumn, W: winter, SP: spring).

4.3.1.2 SIGNAL 2 index

There were similarities between the two SIGNAL methods (with and without weighting factor) for all Brumbys Creek stations over seasons, except for upstream 1 (1) in autumn which changed from mild to moderate pollution when the weighting factor was included (Table 4.3). Generally, upstream stations had similar water quality ratings to that of downstream stations although upstream site scores were qualitatively higher compared to downstream site scores, suggesting the water quality rating downstream showed some recovery. The outlet station was the most impacted, rated as *severe pollution* in three seasons with only spring being an exception (*moderate pollution*).

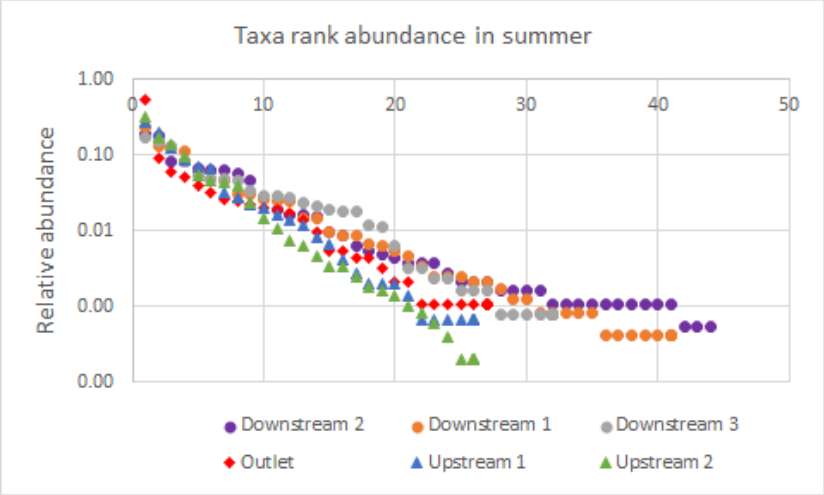
Water quality ratings were consistent over four seasons at upstream 1 (1) (except *mild pollution* in autumn) and the three downstream stations (3, 4 and 5) which all rated as *moderate pollution* (except in summer *mild pollution* for downstream 1 and 3 (3 and 5)). At upstream 2 (2), the water quality rating was slightly better in summer, autumn and spring (*mild pollution*) compared with winter (*moderate pollution*). The water quality rating at the outlet (3) was better in spring (*moderate pollution*) compared to other seasons (*severe pollution*). It would seem the stream conditions at each station did not change substantially over the four sampling times although there were slight differences in site scores between seasons at each station. Generally, site scores at each station were higher in summer before decreasing slightly in autumn and winter and then increasing in spring. This suggests that stream water quality ratings were slightly degraded in autumn and winter before increasing in spring (higher site scores) which showing some recovery. The level of pollution from the most to least impacted, was outlet station (3), downstream 2 (5), downstream 1 (4), downstream 3 (6), upstream 1 (1) and upstream 2 (2).

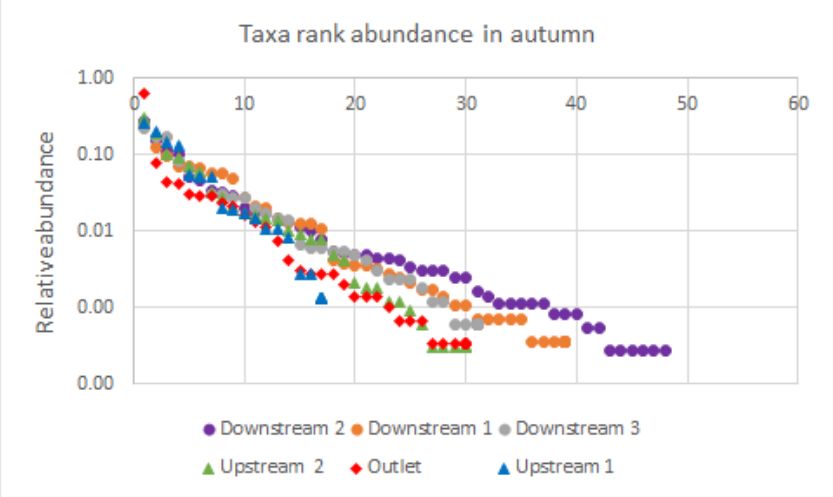
Table 4.3: Water quality rating at six stations in four seasons of 2016 based on SIGNAL 2 scores calculated with and without an abundance weighting factor.

Station	Season	Site score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
Upstream 1 (1)	Summer	4.62	Moderate pollution	4.37	Moderate pollution
Upstream 2 (2)	Summer	5.23	Mild pollution	5.33	Mild pollution
Outlet (3)	Summer	3.93	Severe pollution	3.33	Severe pollution
Downstream 1 (4)	Summer	5.12	Mild pollution	5.17	Mild pollution
Downstream 2 (5)	Summer	4.93	Moderate pollution	4.71	Moderate pollution
Downstream 3 (6)	Summer	5.31	Mild pollution	5.40	Mild pollution
Upstream 1 (1)	Autumn	5.00	Mild pollution	4.84	Moderate pollution
Upstream 2 (2)	Autumn	5.03	Mild pollution	5.27	Mild pollution
Outlet (3)	Autumn	3.93	Severe pollution	3.67	Severe pollution
Downstream 1 (4)	Autumn	4.82	Moderate pollution	4.67	Moderate pollution
Downstream 2 (5)	Autumn	4.88	Moderate pollution	4.65	Moderate pollution
Downstream 3 (6)	Autumn	4.74	Moderate pollution	4.83	Moderate pollution
Upstream 1 (1)	Winter	4.44	Moderate pollution	4.32	Moderate pollution
Upstream 2 (2)	Winter	4.50	Moderate pollution	4.76	Moderate pollution
Outlet (3)	Winter	3.78	Severe pollution	3.26	Severe pollution
Downstream 1 (4)	Winter	4.37	Moderate pollution	4.16	Moderate pollution
Downstream 2 (5)	Winter	4.15	Moderate pollution	4.26	Moderate pollution
Downstream 3 (6)	Winter	4.60	Moderate pollution	4.59	Moderate pollution
Upstream 1 (1)	Spring	4.57	Moderate pollution	4.80	Moderate pollution
Upstream 2 (2)	Spring	5.16	Mild pollution	5.07	Mild pollution
Outlet (3)	Spring	4.00	Moderate pollution	3.45	Moderate pollution
Downstream 1 (4)	Spring	4.41	Moderate pollution	4.43	Moderate pollution
Downstream 2 (5)	Spring	4.40	Moderate pollution	4.69	Moderate pollution
Downstream 3 (6)	Spring	4.95	Moderate pollution	4.91	Moderate pollution

4.3.1.3 Relative abundance

The dominance-diversity curves indicated some dominant taxa at each station (Figure 4.10). Steep dominance-diversity curves occurred at upstream 1, upstream 2, outlet and downstream 3 indicating low diversity with high number of dominant species over four seasons, except for upstream 2 in spring which was flatter in profile indicating a more even abundance. The plots are also flatter at downstream 1 and downstream 2; highlighting a more even distribution of taxa abundance than that of other stations. Furthermore, the downstream stations had slightly variable dominance-diversity curves over four sampling times.





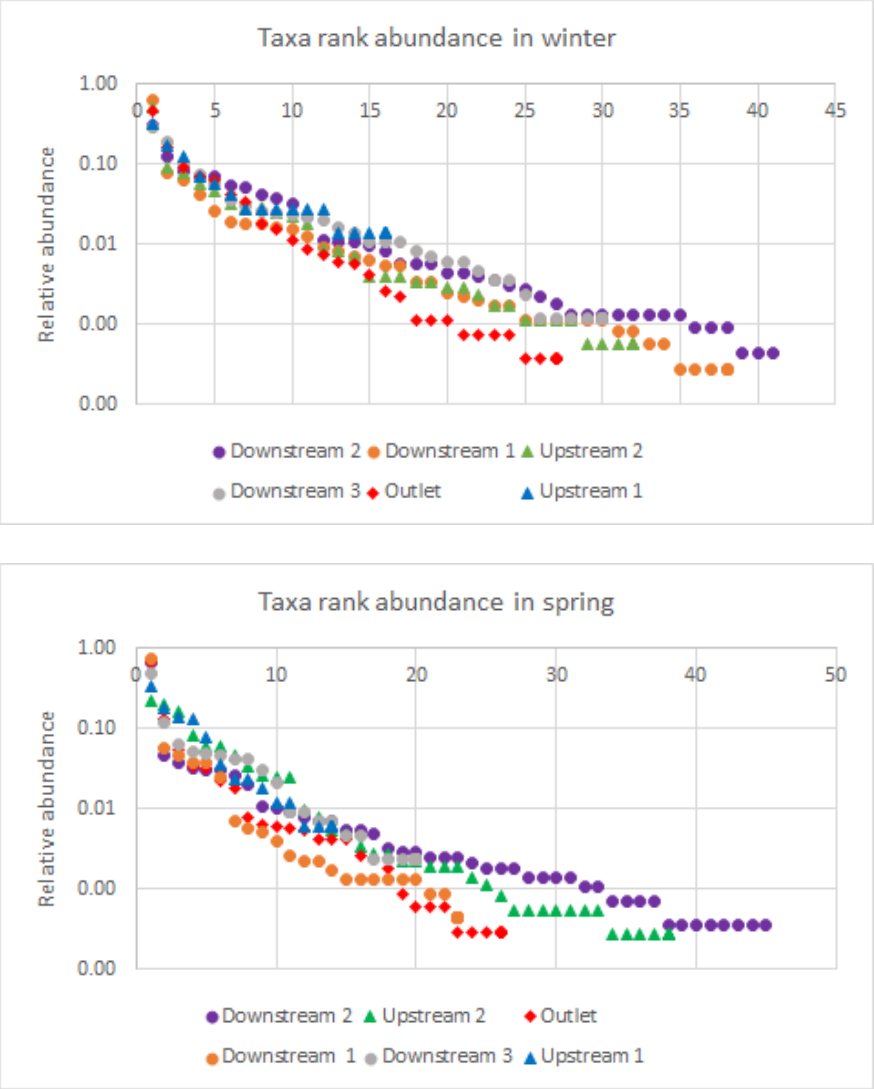
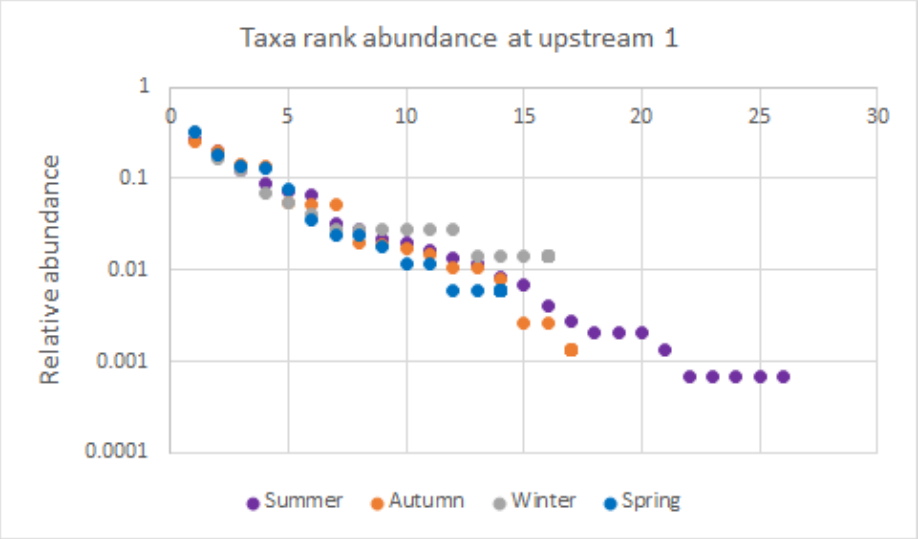


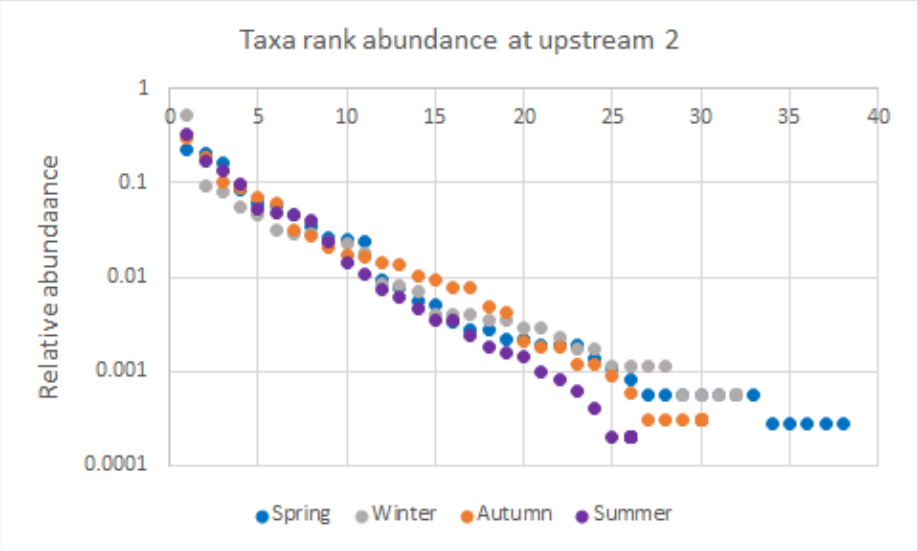
Figure 4.10: The dominance-diversity distribution for 6 stations in each season of 2016

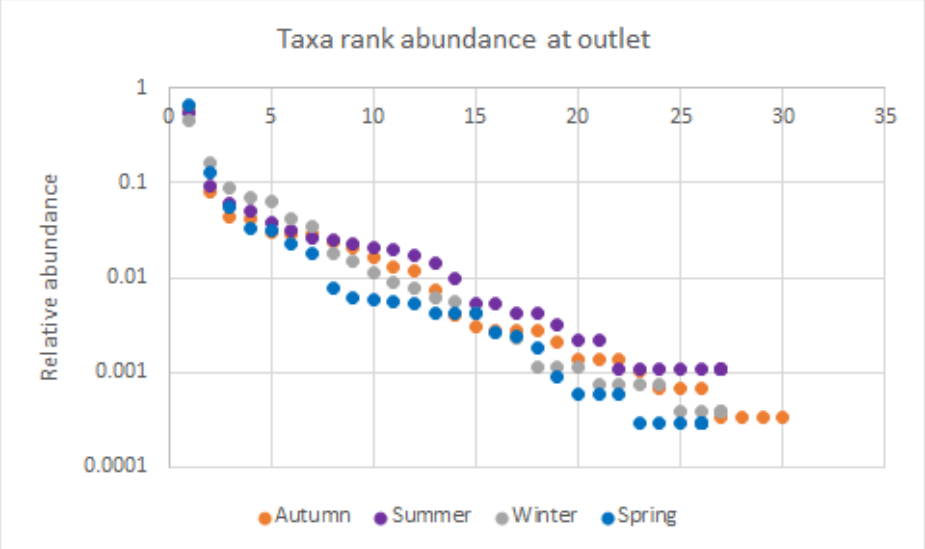
Generally, there were similarities in the dominance-diversity curves of each station among the four sampling times (Figure 4.11) which suggests there were not marked changes in species diversity at each station over seasons. However, there were some exceptions such as upstream 1 in summer, downstream 1 and downstream 3 in spring with steep dominance-diversity curves illustrating low diversity and a high number of dominant species. Within each station, the dominance-diversity curves were quite similar in summer and autumn (except upstream 1) although there were dissimilarities in the dominance-diversity curves in winter and spring at upstream 1, downstream 1 and downstream 3.

Downstream 2 had highest diversity (41 – 48 taxa) while upstream 1 had lowest species diversity (14 – 26 taxa). Moreover, species diversity at the outlet (26 – 30 taxa) and downstream 2 (41 – 48 taxa) were quite stable over four seasons. Species diversity gradually dropped from summer at upstream 1, downstream 1 and downstream 3 whereas there was a gradual increase across time at upstream 2.

The upstream stations were dominated by Hydropsychidae, Caenidae, Paramelitidae, Hydrobiidae, Orthocladiinae and Simulidae (Table 4.4) which are taxa tolerant of pollution; suggesting there was alternative source of impact on the river stream at these control sites. Furthermore, Hydropsychidae, Chironominae, Tanypodinae, Orthocladiinae, Simulidae, Caenidae, Paramelitidae and Oligochaeta are taxa tolerant to very tolerant of pollution and were dominant at the outlet and downstream stations. Oligochaeta was the most abundant taxa at the outlet (from 0.45 to 0.66 in relative abundance); and was the key species differentiating this station from other stations. Downstream 3 seemed to have some similar taxa to upstream stations, suggesting similar conditions.


Taxa rank abundance at upstream 1
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
Summer
Autumn
Winter
Spring


Taxa rank abundance at upstream 2
Relative abundaance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Spring
Winter
Autumn
Summer


Taxa rank abundance at outlet
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
Autumn
Summer
Winter
Spring

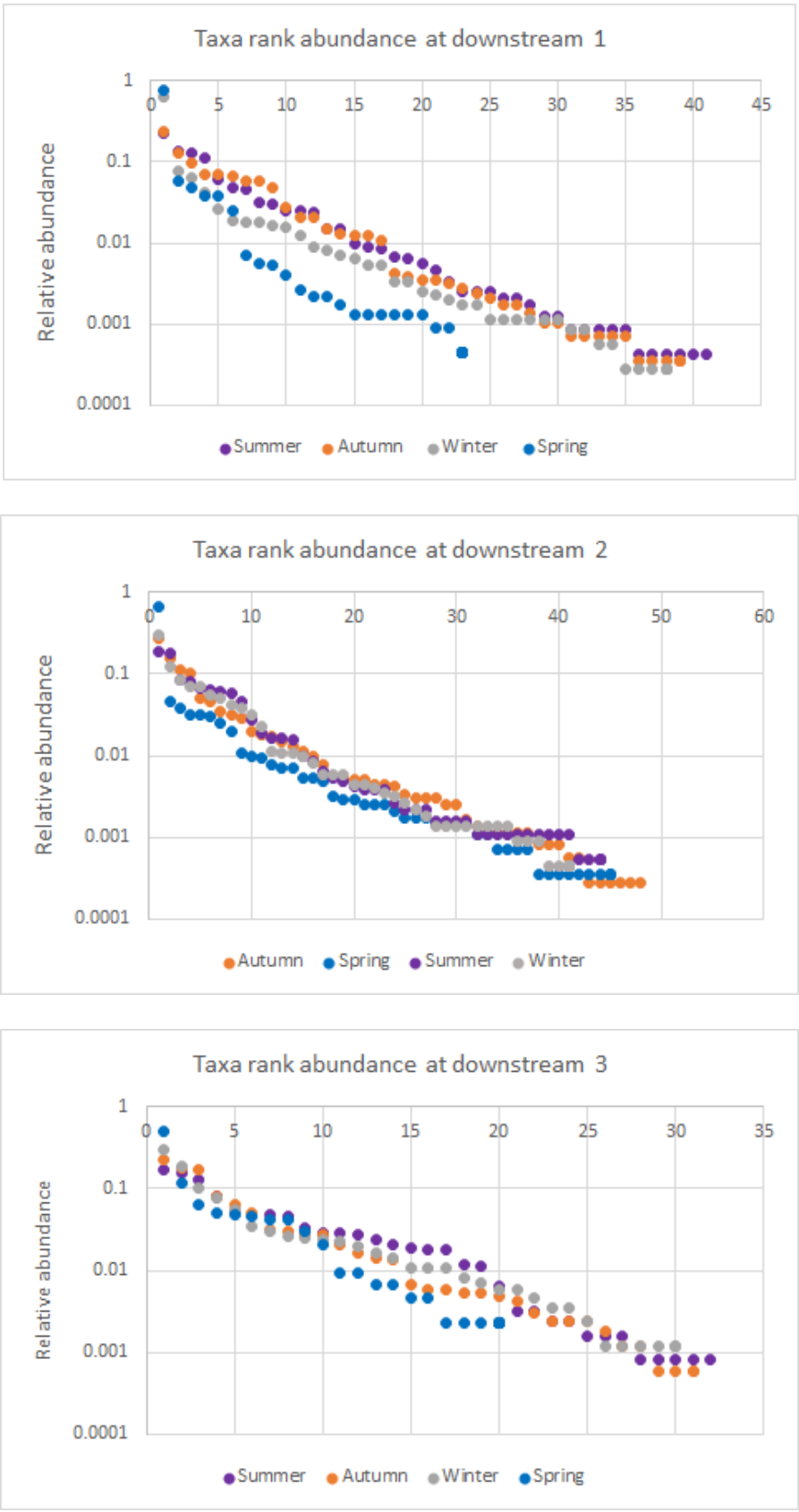


Figure 4.11: The dominance-diversity distribution for 4 seasons at each station in Brumbys Creek

Table 4.4: The three most dominant taxa and their relative abundance at each station over the four seasons in Brumbys Creek. Station and season abbreviations as in Figure 4.4

Station/Season	Total N. of taxa	First 3 dominated taxa	Relative abundance	Individuals of each taxa/ total individuals
1S	26	Hydropsychidae	0.27	404/1470
		Caenidae	0.20	298/1470
		Paramelitidae	0.12	179/1470
1A	17	Caenidae	0.26	194/745
		Hydropsychidae	0.20	147/745
		Hydrobiidae	0.14	106/745
1W	16	Orthocladiinae	0.32	23/72
		Chironominae	0.17	12/72
		Paramelitidae	0.13	9/72
1SP	14	Orthocladiinae	0.33	55/167
		Simulidae	0.18	30/167
		Caenidae	0.14	23/167
2S	26	Paramelitidae	0.33	1627/4971
		Hydropsychidae	0.17	854/4971
		Hydrobiidae	0.14	681/4971
2A	30	Hydropsychidae	0.30	985/3321
		Hydrobiidae	0.19	615/3321
		Paramelitidae	0.10	331/3321
2W	32	Hydrobiidae	0.51	897/1743
		Hydropsychidae	0.09	158/1743
		Paramelitidae	0.08	137/1743
2SP	38	Hydrobiidae	0.22	809/3626
		Caenidae	0.20	741/3626
		Oligochaeta	0.16	579/3626
3S	27	Oligochaeta	0.54	504/933
		Chironominae	0.09	85/933
		Tanypodinae	0.06	57/933
3A	30	Oligochaeta	0.63	1858/2945
		Orthocladiinae	0.08	235/2945
		Simulidae	0.04	127/2945
3W	27	Oligochaeta	0.45	1184/2631
		Chironominae	0.16	425/2631
		Orthocladiinae	0.09	236/2631
3SP	26	Oligochaeta	0.66	2224/3384
		Chironominae	0.13	436/3384
		Orthocladiinae	0.05	183/3384

4S	41	Simulidae	0.23	543/2366
		Paramelitidae	0.13	314/2366
		Caenidae	0.13	299/2366
4A	39	Oligochaeta	0.24	685/2868
		Simulidae	0.13	366/2868
		Paramelitidae	0.10	275/2868
4W	38	Oligochaeta	0.62	2210/3550
		Paramelitidae	0.08	274/3550
		Simulidae	0.06	229/3550
4SP	23	Oligochaeta	0.75	1720/2297
		Caenidae	0.06	131/2297
		Paramelitidae	0.05	107/2297
5S	44	Oligochaeta	0.19	348/1864
		Caenidae	0.18	340/1864
		Paramelitidae	0.08	155/1864
5A	48	Cladocera	0.27	970/3611
		Oligochaeta	0.15	550/3611
		Caenidae	0.11	396/3611
5W	41	Oligochaeta	0.30	680/2241
		Chironominae	0.13	281/2241
		Paramelitidae	0.08	186/2241
5SP	45	Oligochaeta	0.67	1900/2827
		Chironominae	0.05	130/2827
		Hydrobiidae	0.04	107/2827
6S	32	Caenidae	0.17	212/1252
		Hydropsychidae	0.16	195/1252
		Baetidae	0.13	162/1252
6A	31	Caenidae	0.22	371/1675
		Orthocladiinae	0.17	293/1675
		Oligochaeta	0.17	290/1675
6W	30	Oligochaeta	0.29	249/853
		Caenidae	0.19	159/853
		Elmidae (L)	0.10	85/853
6SP	20	Oligochaeta	0.49	215/437
		Caenidae	0.12	51/437
		Oniscigastridae	0.06	28/437

4.3.1.4 Total abundance, taxa richness, Simpson diversity index

The effect of station on the total abundance, taxa richness and Simpson's diversity of macroinvertebrates interacted with season (PERMANOVA, $F_{15,48}$=6.42, 2.14, 4.47; P_{MC}=0.0001, 0.0243, 0.0001 respectively, Figures 4.12-4.14). There was a lower total abundance and taxa richness at upstream 1 and downstream 3 than at other stations whereas the outlet and downstream 1 had lower diversity index compared to other stations. The outlet appears to be the most impacted station with higher total abundance, but lower taxa richness and diversity index compared to upstream stations and the furthest downstream station (downstream 3).

For total abundance, upstream 1 differed significantly from other stations over four seasons (PERMANOVA, Table 4.5). In summer, the upstream 2 station (1657 individuals) had a significantly higher total abundance than other stations and was three times higher than upstream 1 (490 individuals) while the outlet in summer had the lowest total abundance (289 individuals) but was only significantly lower than upstream 2. Total abundance then increased to 789 individuals at downstream 1 before decreasing to 621 and 417 individuals at downstream 2 and downstream 3 respectively. In the other three seasons (autumn, winter and spring), upstream 1 had a significantly lower total abundance (248, 24, 57 individuals), followed by downstream 3 (559, 364, 145 individuals); and both were lower than other stations. Total abundance in winter was generally lower than the other seasons. Moreover, there were no significant differences in total abundance between four downstream stations in summer and autumn but there were in winter and spring (Table 4.5). With the exception of autumn, total abundance increased gradually from the outlet to downstream 1 before declining at downstream 2 and downstream 3. Looking at each station, there were similarities

in total abundance between four seasons at downstream 2. A similar abundance between summer and autumn was seen at upstream 1, outlet, downstream 1 and downstream 2 while there was similar total abundance between winter and spring at upstream 1 and outlet.

There was a significant station x season interaction for taxa richness although generally, a similar trend existed between stations over the four seasons (Figure 4.13, Table 4.5). Taxa richness was significantly lower at upstream 1 (18 taxa in summer, 13 in autumn, 11 in winter, 10 in spring) compared to all other stations (Table 4.5). Generally, the total number of taxa tended to increase at upstream 2, outlet, downstream 1 and downstream 2; and downstream 2 had the highest number of taxa in all seasons except spring. However, taxa richness of downstream 3 decreased and seemed to be just slightly higher than the upstream 1. No significant differences in taxa richness between summer and autumn were seen at all stations while taxa richness at upstream 1 between winter and spring were not significantly different. There were also no significant differences in taxa richness between four seasons at the upstream 2, outlet and downstream 2. Only downstream 1 and downstream 3 had significant differences in taxa richness between winter and spring.

Figure 4.12: Mean (± SE; n=3 replicates) of total abundance of macroinvertebrates in six stations at Brumbys over four seasons.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

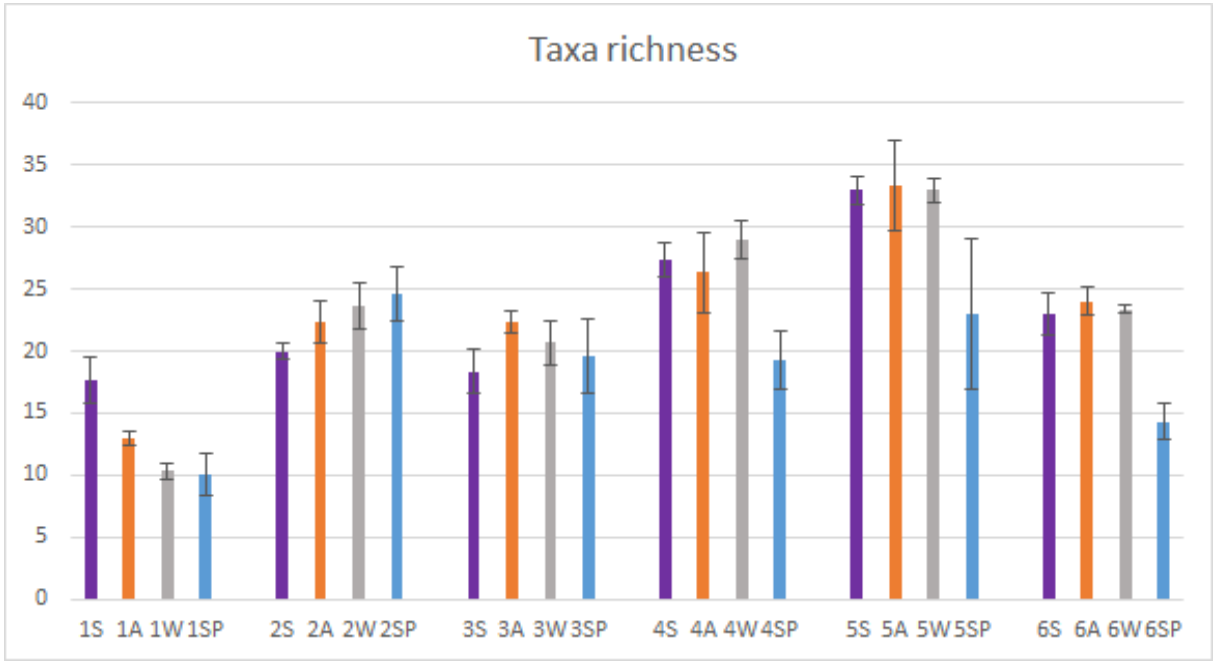


Figure 4.13: Mean (±SE; n=3 replicates) of taxa richness of macroinvertebrates in six stations at Brumbys over four seasons.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

There was a significant station x season interaction for Simpson diversity index (Table 4.5) and generally, higher diversity was observed at the three downstream stations compared to the outlet and upstream stations. Spring had the lowest values, ranging from 1.68 to 3.68 with a similar trend between stations. In summer and autumn, the two upstream stations had higher diversity indices than the outlet station (lowest diversity index); but lower diversity indices than the three downstream stations. In contrast, downstream 1 had the lowest diversity in winter and spring (2.5 and 1.68 respectively), followed by upstream 2 (3.4 and 3.25) and outlet (4.46 and 2.5) whereas diversity index at upstream 1, downstream 2 and downstream 3 were higher than other stations. Moreover, there were no significant differences between seasons at upstream 1 and the outlet while diversity indices of other stations were not significantly different in summer and autumn which also were much higher than in spring.

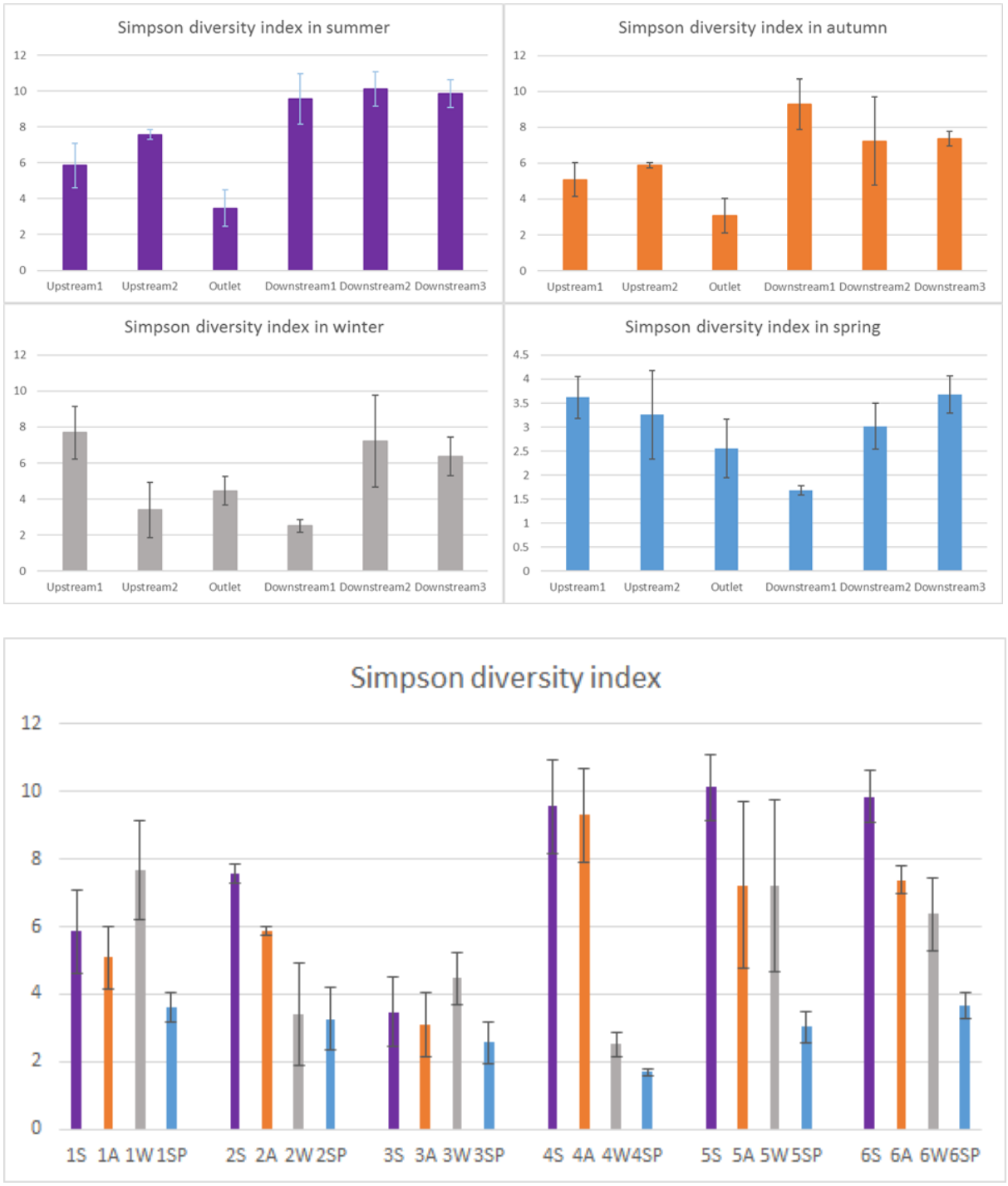


Figure 4.14: Mean (±SE; n=3 replicates) of Simpson diversity index of macroinvertebrates in six stations at Brumbys over four seasons.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

Table 4.5: ANOVA testing Station (St), Season (Se) and Station x Season (StxSe) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data square root transformed. Permutations (N=9,999) were applied to the residuals under a reduced mode. Pair-wise post hoc comparisons were done within the station x season interaction. Station and season abbreviations as in Figure 4.4

Source	Df	P_{seudo}-F	P (MC)	Post hoc comparison	P_{seudo}-F	P (MC)	Post hoc comparison
		Total abundance			Taxa richness		
Transformation		Square root			Square root		
St	5	38.588	0.0001		30.24	0.0001	
Se	3	3.2223	0.0288		7.1071	0.0003	
StxSe	15	6.4221	0.0001	2S ≠ others 1A ≠ 2A, 5A, 6A 2A ≠ 6A 1W ≠ others 4W ≠ 2W, 5W, 6W 1SP ≠ others 2SP, 3SP≠ 4SP, 6SP 4SP≠ 5SP ≠ 6SP 1S = 1A; 1W = 1SP 2SP = 2S, 2A 3A = 3S, 3W, 3SP; 3W = 3SP 4S = 4A = 4W 5S = 5A = 5W = 5SP 6S = 6A = 5W	2.1402	0.0243	1S, 2S, 3S ≠ 4S, 5S 5S ≠ 4S, 6S 1A ≠ others 3A ≠ 5A 1W ≠ others 3W, 6W ≠ 4W, 5W 1SP ≠ 2SP, 3SP, 4SP 2SP ≠ 6SP 1S = 1A; 1SP = 1A, 1W 2S = 2A = 2W = 2SP 3S = 3A = 3W = 3SP 4S = 4A, 4W, 4SP 4A = 4W, 4SP 5S = 5A = 5W = 5SP 6S = 6A = 6W
Residuals	28						
		Simpson diversity index					
Transformation		Square root					
St	5	6.5578	0.0005				
Se	3	15.406	0.0001				
StxSe	15	4.4662	0.0001	2S ≠ 4S, 5S 3S ≠ 4S, 5S, 6S 2A ≠ 3A, 6A 3A ≠ 4A, 6A 1W, 6W ≠ 2W, 4W 1SP ≠ 3SP, 4SP, 5SP, 6SP 2SP ≠ 3SP, 4SP, 5SP 4SP ≠ 6SP 1S = 1A = 1W = 1SP 2S = 2A = 2SP 3S = 3A = 3W = 3SP 4S = 4A; 4W = 4SP 5A = 5W = 5SP 6W = 6A, 6S, 6SP			
Residuals	48						

1: upstream1, 2: upstream2, 3: outlet, 4: downstream1, 5: downstream2, 6: downstream3
S: summer, A: autumn, W: winter, SP: spring

4.3.2 Differences in the macroinvertebrate community between seven stations in the Florentine in summer and autumn over two years (2016 and 2017)

4.3.2.1 Assessment of macroinvertebrate assemblages over four seasons

Principal CO analysis of the Florentine data clearly separates the two upstream stations (1 and 2) from all downstream stations as well as the outlet (3) and station just below outlet (4) from other further downstream stations (5, 6 and 7, Figure 4.15). The outlet (3) and downstream 1 (4) stations would appear to be the most different from all of other stations over four sampling times. This suggested that the closer the station was to the effluent, the greater the impact. The first and second PCOs both contribute to the separation of stations and also seasons (PERMANOVA, $F_{18,56}$= 3.62, P_{MC}= 0.0001) and macroinvertebrate composition was significantly different between every pair of stations (pairwise PERMANOVA, P<0.05) except the two upstream stations (1 and 2) and downstream 1 (4). The first two PCO axes accounted for 49.1% of the variation, and show a separation of the upstream stations (1 and 2) from outlet (3) and downstream stations (4, 5, 6 and 7) as well as of the outlet (3) and downstream 1 (4) from other three downstream stations (5, 6 and 7). Moreover, there were similarities in community assemblages between upstream 1 (1) and uptream 2 (2) as well as between outlet (3) and downstream 1 (4) whereas downstream 2, 3 and 4 (5, 6 and 7) group together and are not easily distinguishable from one another. Vectors loadings indicate Psephenidae, Gripopterygidae, *Eusthenia costalis*, Ceinidae, Elmidae (A), Baetidae, Paramelitidae and Leptoplebiidae were positively correlated with PCO1 and most strongly associated with communities of upstream stations (1 and 2); indicating non-farming conditions (Figure 4.15). Oligochaeta, Planorbidae, *Physa acuta*, Hirudinae and Ancylidae were positively correlated with PCO2, and those taxa were also abundant at the oulet (3) and downstream 1 (4) stations; indicatiing impacted conditions. Orthocladiinae, Tanypodinae, Chironominae and

Hydropsychidae were negatively correlated with both PCO axes and highly associated with the further downstream stations (5, 6 and 7); which can be used as indicators of downstream conditions.

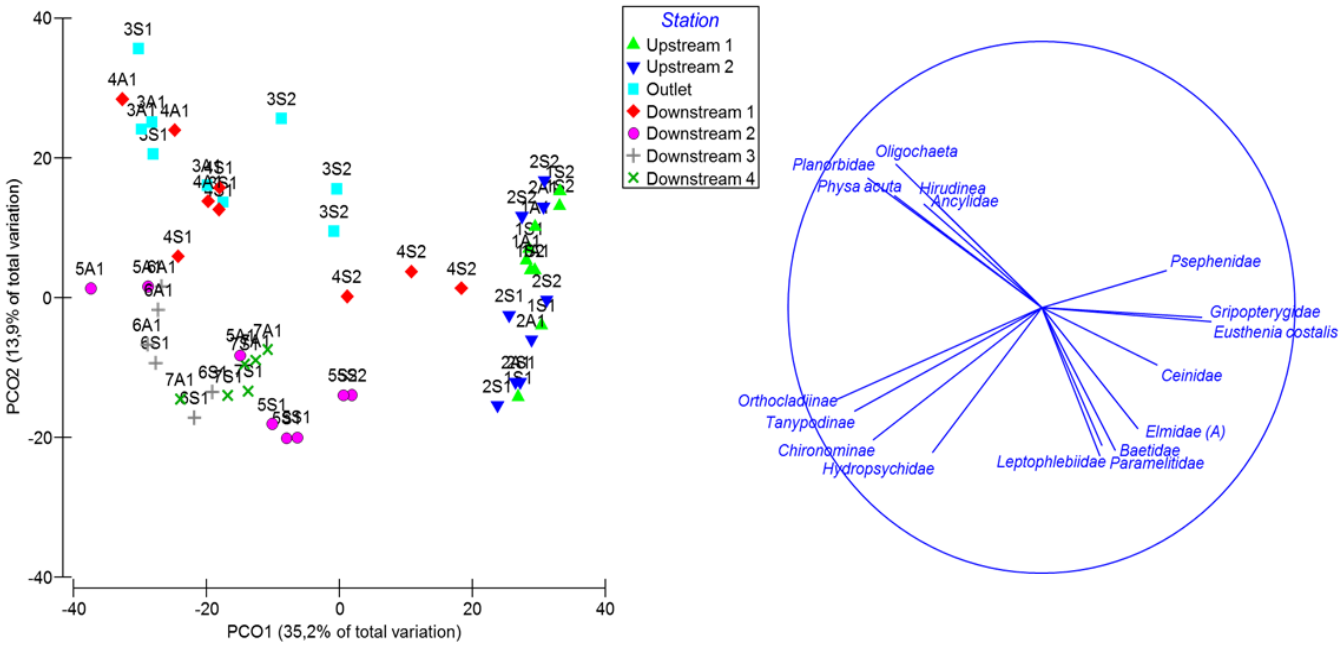


Figure 4.15: Two dimensional PCO plot for macroinvertebrate matrix fauna of seven stations at the Florentine river over four sampling times. Fitted macroinvertebrate fauna vectors based on Pearson correlation (>0.5) indicate contribution of taxa to dissimilarity between stations
(Numbers refer to 1: upstream1, 2: upstream2, 3: outlet, 4: downstream1, 5: downstream2, 6: downstream3, 7: downstream4) (Letters refer to S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017)

Table 4.6: SIMPER analyses showing the relative taxa contributions (%) to station (AS: average similarity between three replicates)

Rank	Upstream 1 (1) (AS = 73.54)	Upstream 2 (2) (AS = 65.33)	Outlet (3) (AS = 76.29)	Downstream 1(4) (AS = 68.83)	Downstream 2 (5) (AS = 83.45)	Downstream 3 (6) (AS = 73.83)	Downstream 4 (7) (AS=80.29)
1	Baetidae 17.3	Baetidae 19.72	Oligochaeta 20.15	Oligochaeta 14.08	Orthocladiinae 12.31	Orthocladiinae 12.36	Hydropsychidae 12.85
2	Costora delora 11.12	Leptophlebiidae 8.26	Planorbidae1 10.33	Orthocladiinae 11.78	Baetidae 11.94	Hydropsychidae 12.32	Baetidae 11.82
3	Eusthenia costalis 8.07	Lingora sp. 7.08	Orthocladiinae 8.29	Baetidae 10.89	Chironominae 9.26	Chironominae 11.86	Orthocladiinae 9.61
4	Leptophlebiidae 6.42	Hydropsychidae 6.58	Baetidae 7.68	Tanypodinae 8.98	Tanypodinae 8.96	Baetidae 10.45	Tanypodinae 8.6
5	Elmidae (L) 5.82	Gripopterygidae 6.56	Tanypodinae 7.54	Chironominae 8.6	Hydropsychidae 8.76	Tanypodinae 8.62	Chironominae 8.24
6	Hydropsychidae 5.82	Eusthenia costalis 6.13	Physa acuta 6.65	Planorbidae1 5.55	Leptophlebiidae 7.1	Leptophlebiidae 6.8	Leptophlebiidae 6.24
7	Psephenidae 4.76	Elmidae (L) 4.9	Chironominae 5.87	Leptophlebiidae 5.32	Elmidae (L) 5.11	Costora delora 4.54	Costora delora 5.2
8	Conoesucidae3 4.46	Psephenidae 4.76	Elmidae (L) 4.19	Costora delora 5.3	Oligochaeta 3.63	Physa acuta 3.63	Elmidae (L) 4.33
9	Leptoceridae2 3.98	Conoesucidae3 4.55	Leptophlebiidae 3.36	Hydropsychidae 4.07	Elmidae (A) 3.19	Planorbidae1 3.59	Oligochaeta 3.57
10	Gripopterygidae 3.75	Leptoceridae2 3.04	Hydropsychidae 2.87	Elmidae (L) 3.15	Simulidae 3	Elmidae (L) 3	Conoesucidae3 3.25
11	Lingora sp. 3.59	Elmidae (A) 2.87	Psephenidae 2.59	Eusthenia costalis 2.87	Conoesucidae3 2.67	Oligochaeta 2.55	Hydrobiosidae4 3.03
12	Elmidae (A) 3.52	Conoesucidae 2.83	Conoesucidae3 2.37	Physa acuta 2.67	Planorbidae1 2.18	Ecnomidae 2.44	Eusthenia costalis 2.44
13	Paramelitidae 3.12	Scirtidae 2.74	Elmidae (A) 1.6	Conoesucidae3 2.58	Costora delora 2.11	Conoesucidae3 1.98	Lingora sp. 2.39
14	Conoesucidae1 2.92	Conoesucidae1 2.48	Leptoceridae2 1.49	Psephenidae 2.19	Leptoceridae2 2.1	Paramelitidae 1.84	Elmidae (A) 2.33
15	Hydrobiosidae4 2.64	Helicopsychidae 2.34	Costora delora 1.33	Leptoceridae2 2.11	Lingora sp. 1.87	Elmidae (A) 1.72	Leptoceridae2 2.08
16	Ceinidae 2.6	Costora delora 2	Eusthenia costalis 1.33		Gripopterygidae 1.77	Lingora sp. 1.62	Paramelitidae 1.96
17	Helicopsychidae 1.71	Paramelitidae 1.93	Paramelitidae 1.3		Paramelitidae 1.6	Leptoceridae2 1.57	Hydrobiosidae3 1.33

SIMPER analysis support this assessment and shows clear differences in the key taxa differentiating uptream, outlet and downstream communities (Table 4.6). The differentiation mainly resulted from the presence of taxa at downstream locations that were largely absent from upstream locations; which reflected the changes in communities. Oligochaeta was a key taxa which played an important role in differentiating between stations and was absent upstream, at high abundance at the outlet (3) and downstream 1 (4), and at low abundance at further downstream stations (5, 6 and 7). Futhermore, similar communities was seen between upstream 1 (1) and upstream 2 (2), between outlet (3) and downstream 1 (4), and between the three further downstream stations (5, 6 and 7). Therefore in terms of impact, the results sugggest level of pollution of outlet (3) was greater than downstream 1 (4), followed by downstream 2 (5), downstream 3 (6) and downstream 4 (7). The two upstream stations (1 and 2) appeared to be unimpacted.

4.3.2.1.1 Assessment of macroinvertebrate assemblages at all stations for each single season in Florentine river

PCO in each season showed that there was a different pattern in summer 2017 after the major flood in winter 2016 (Figure 4.16 – 4.19). Generally over the four sampling times, upstream stations (1 and 2) consistently separated out along PCO1 whereas outlet (3) and downstream 1 (4) separated along PCO2. Furthermore, the cluster analysis showed an upstream to downstream gradient over four sampling times. In summer and autumn 2016, the outlet (3) and downstream stations (4, 5, 6 and 7) were largely separated from upstream stations (1 and 2) along PCO1 and PCO2. However, there is less defined separation between downstream and upstream stations along PCO2 in summer 2017, highlighting the community changes in summer 2017 after the flood. Communities recorded at all stations in summer 2017 differed from summer and autumn 2016; suggesting the flood influenced stream community

composition. The pattern in autumn 2017 then changed back to being more similar to summer and autumn 2016.

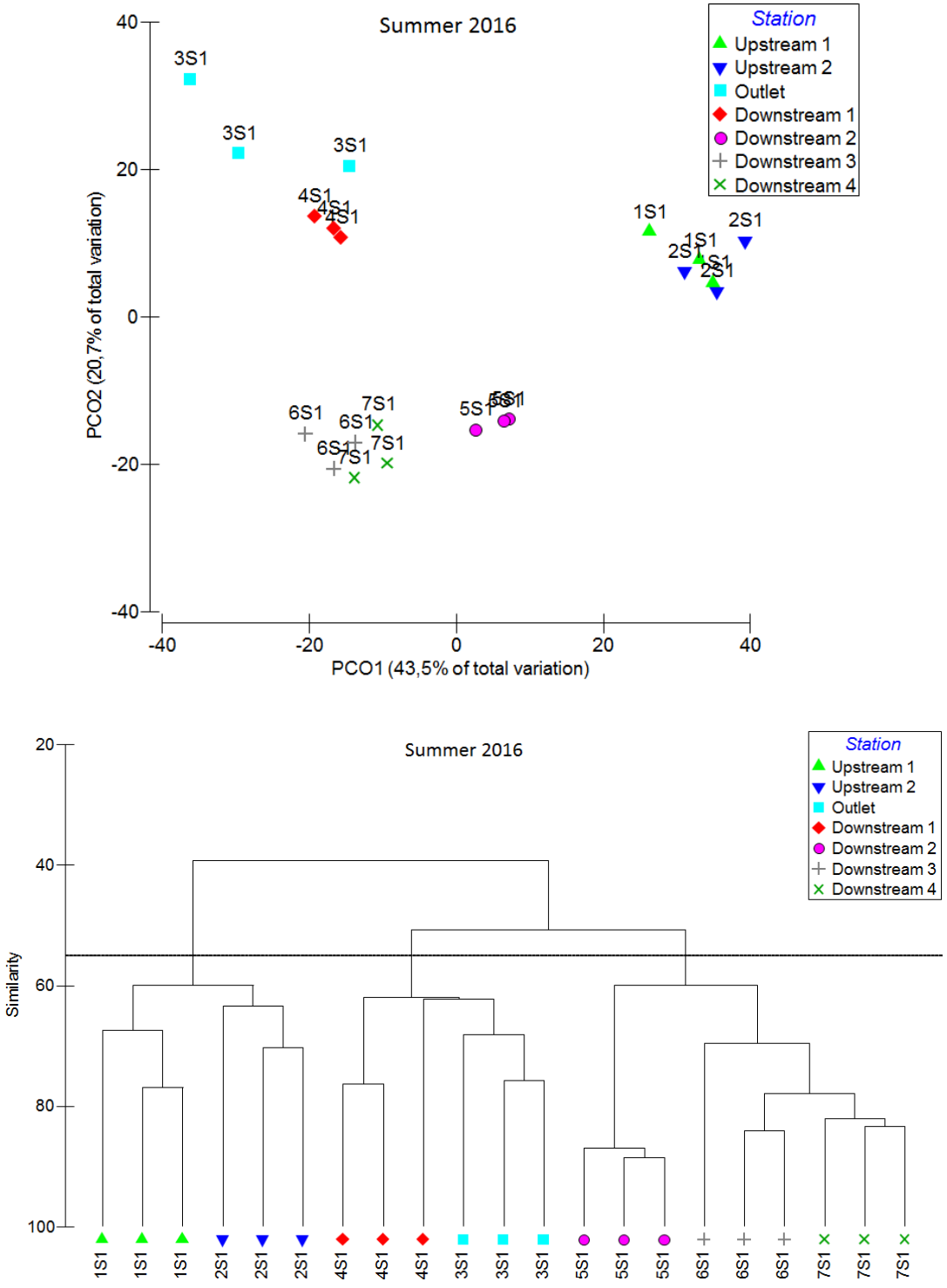


Figure 4.16: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of seven stations at the Florentine in summer 2016. Station abbreviations as in Figure 4.15

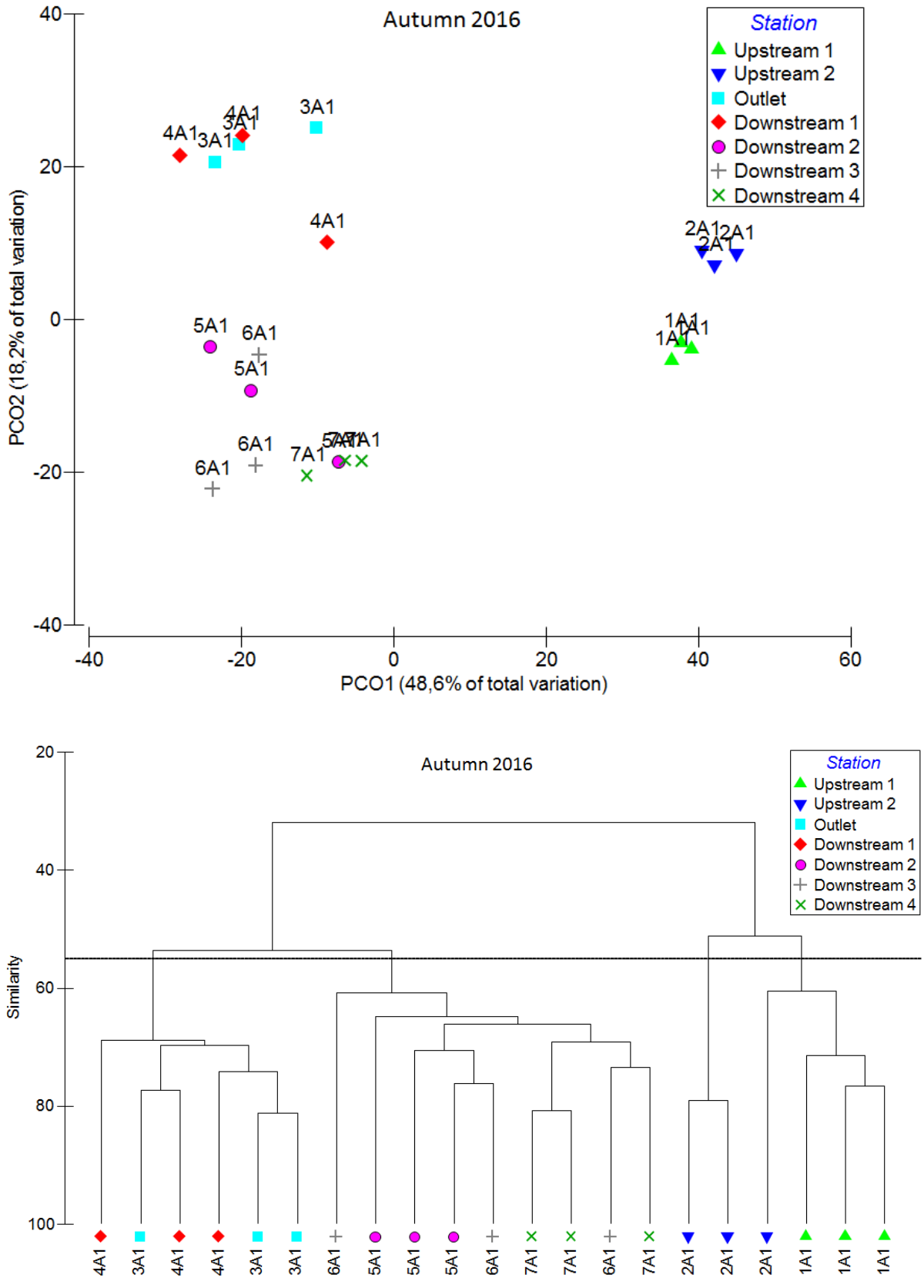


Figure 4.17: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of seven stations at the Florentine in autumn 2016. Station abbreviations as in Figure 4.15

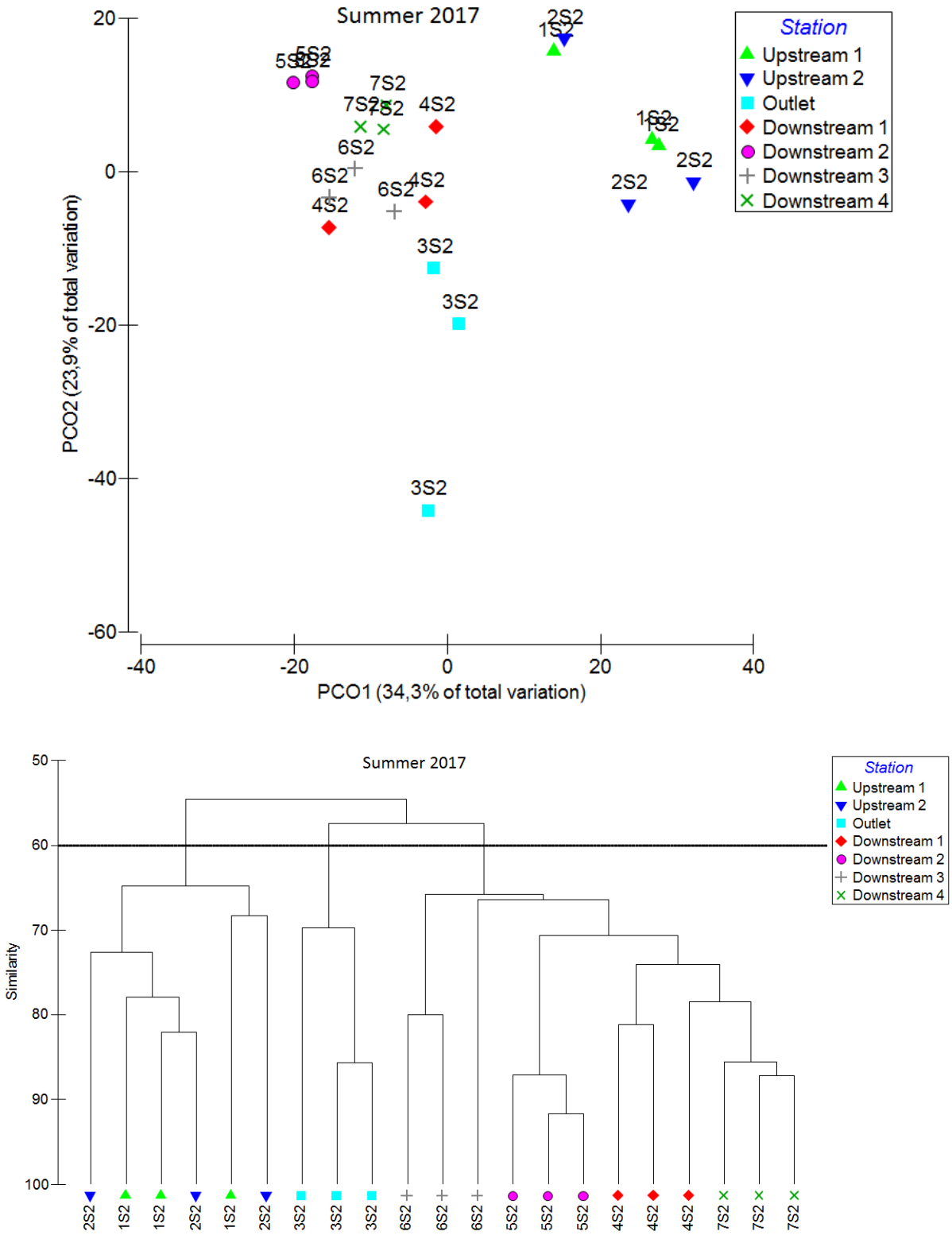


Figure 4.18: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of seven stations at the Florentine in summer 2017. Station abbreviations as in Figure 4.15

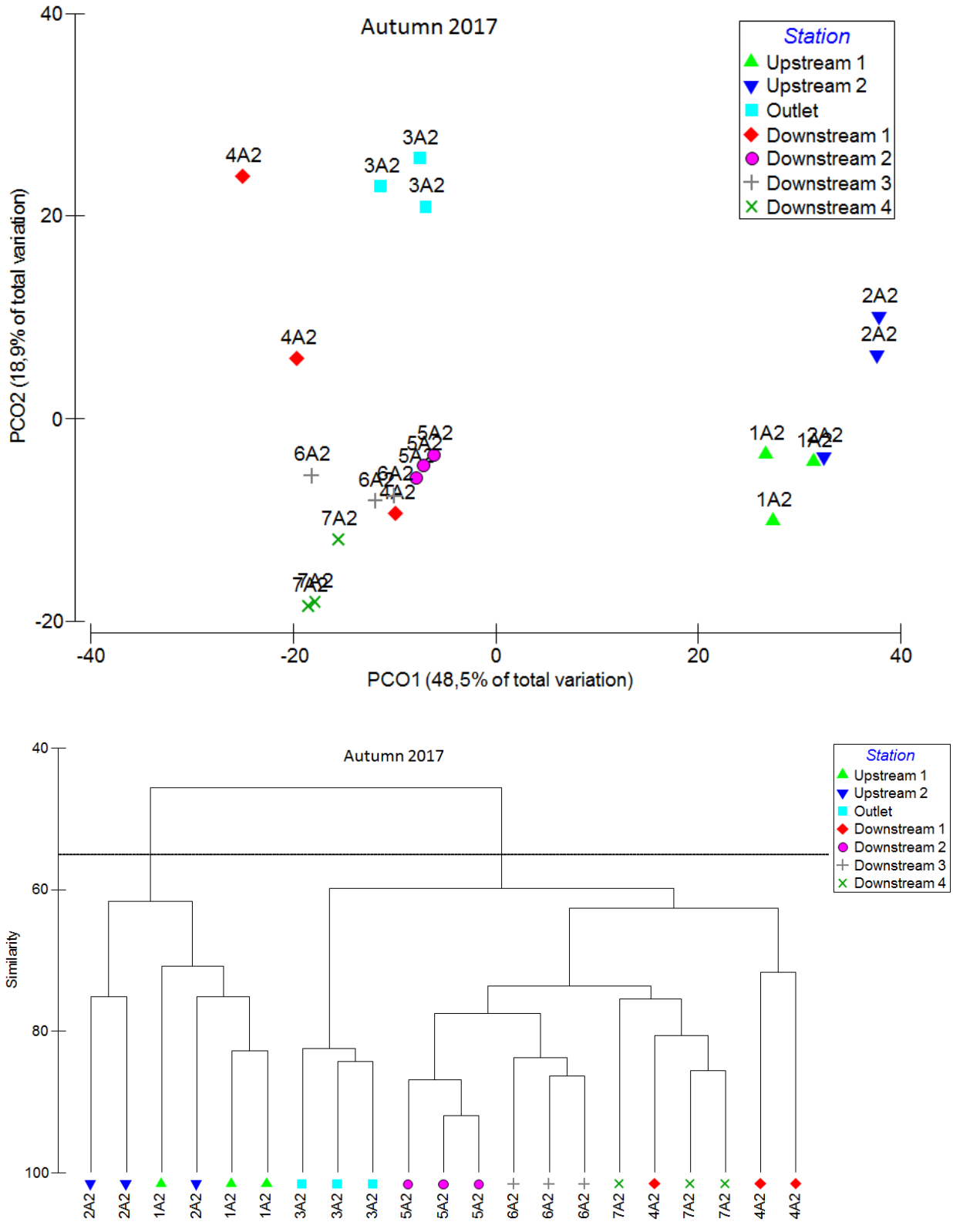
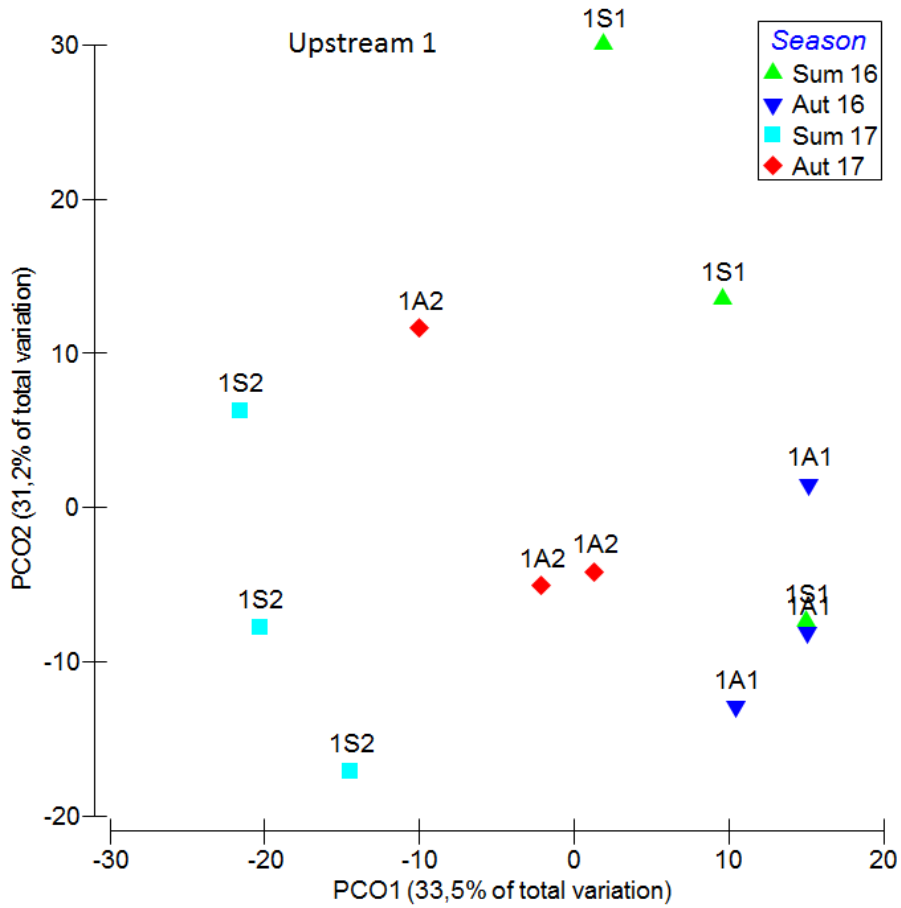
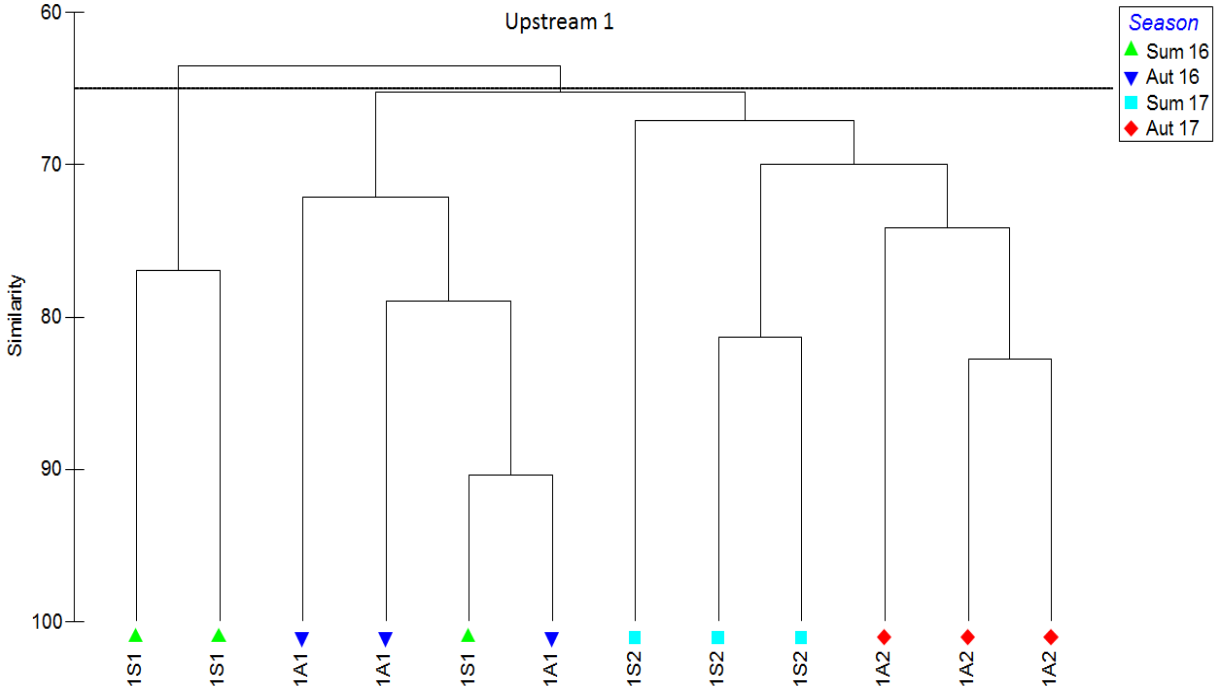


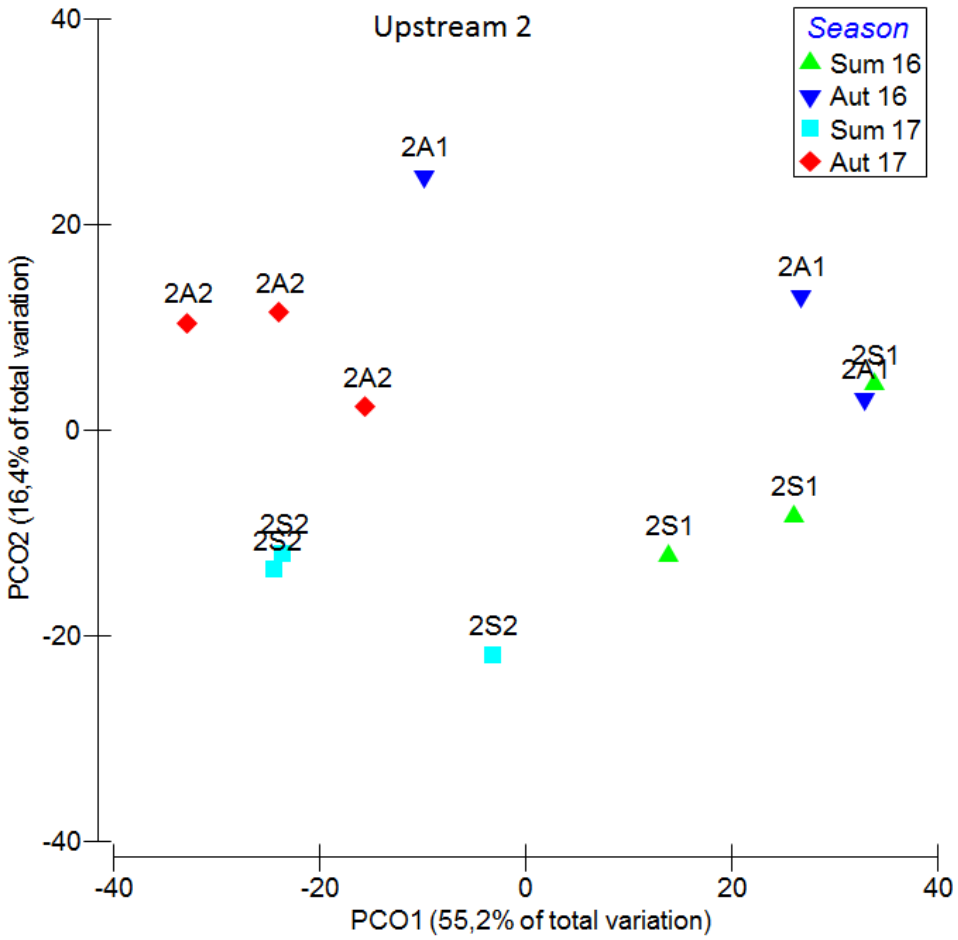
Figure 4.19: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of seven stations at the Florentine in autumn 2017. Station abbreviations as in Figure 4.15

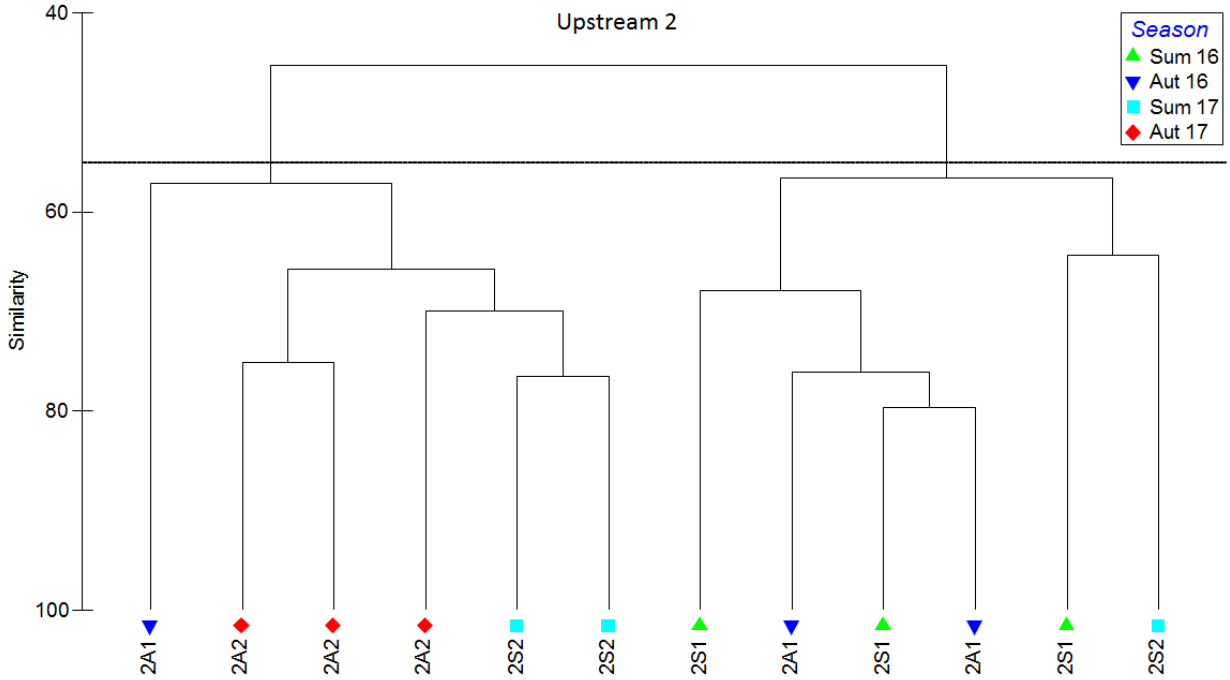
4.3.2.1.2 Assessment of macroinvertebrate assemblages at each station in the Florentine over four seasons

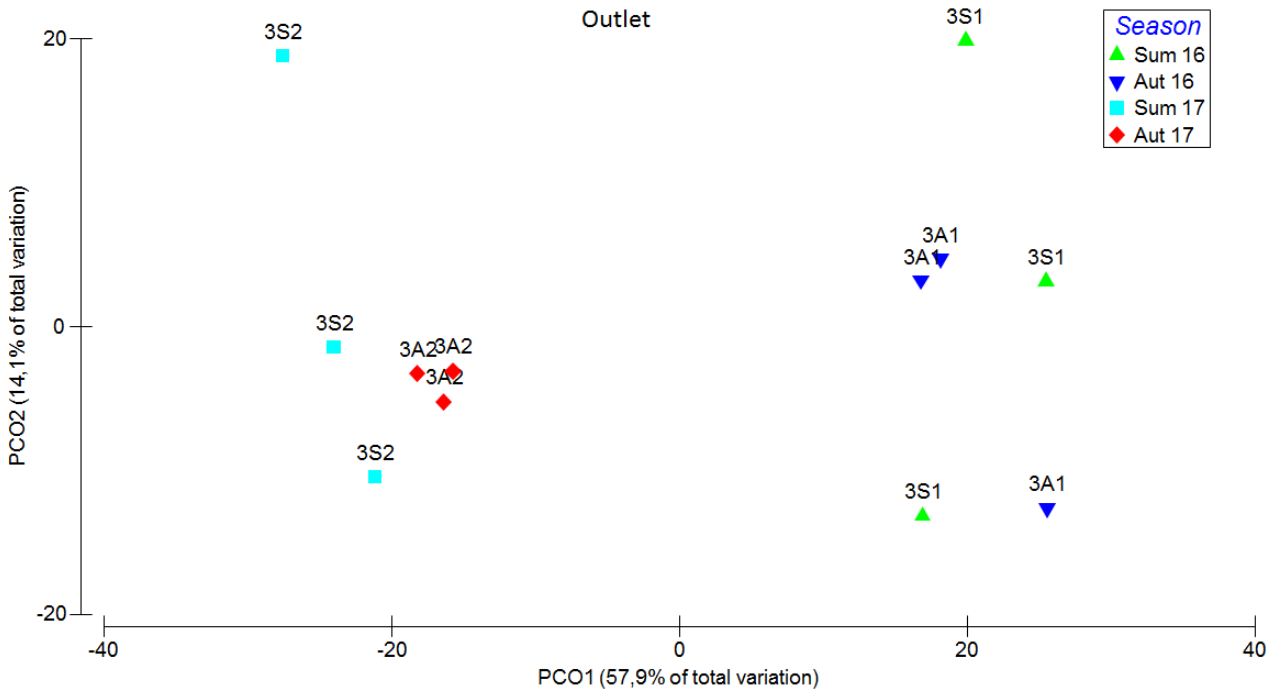
Figure 4.20 illustrates that the stations in the Florentine were often separated by year (2016 vs. 2017) rather than season. Particularly, the communities were more similar in summer and autumn 2016, and in summer and autumns 2017 rather than summer 2016 and 2017 or autumn 2016 and autumn 2017. The trend was similar for all stations, except downstream 3 (6) and downstream 4 (7). These two further downstream stations (6 and 7) had more similar communities in summer 2016, autumn 2016 and autumn 2017. However, the communities in summer 2017 at downstream 3 and 4 (6 and 7) were different from other seasons, especially autumn 2017, suggesting the flood in winter 2016 influenced macroinvertebrate community at all stations.

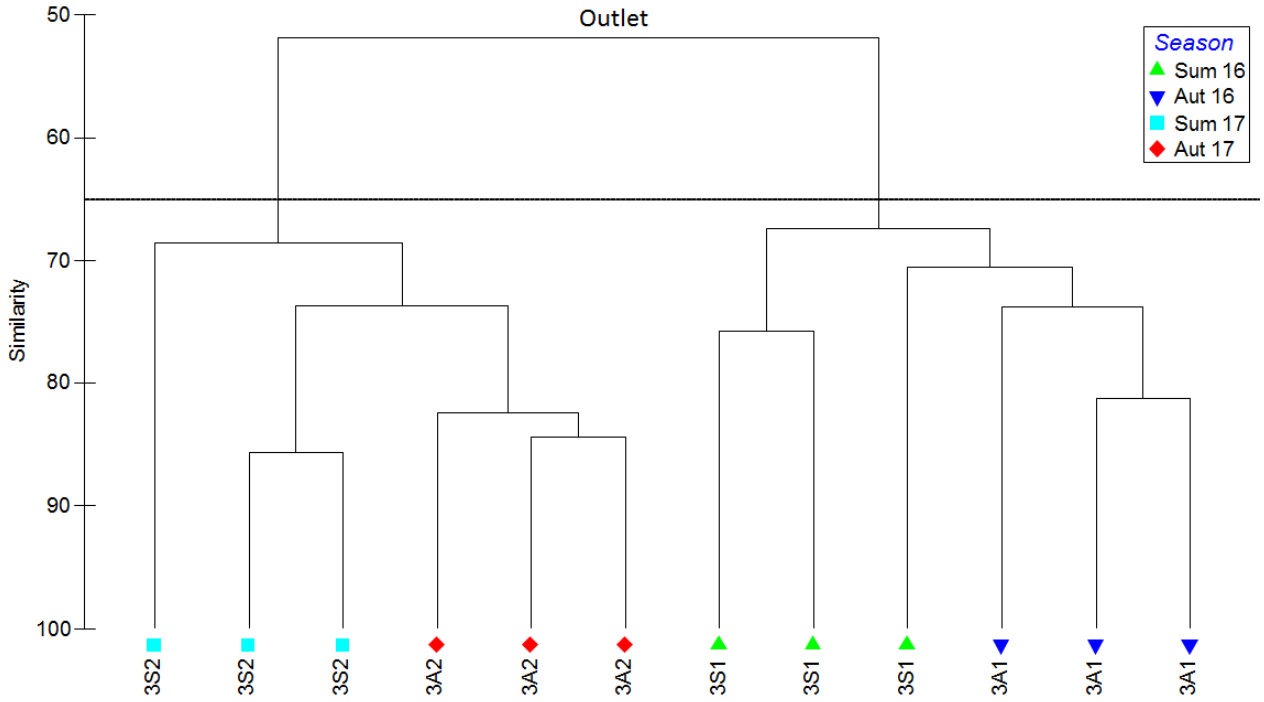

Upstream 1
Season
Sum 16
Aut 16
Sum 17
Aut 17
PCO2 (31,2% of total variation)
PCO1 (33,5% of total variation)
30
20
10
0
-10
-20
-30
-20
-10
0
10
20
1S1
1S1
1S1
1A2
1A2
1A2
1S2
1S2
1S2
1A1
1A1
1A1

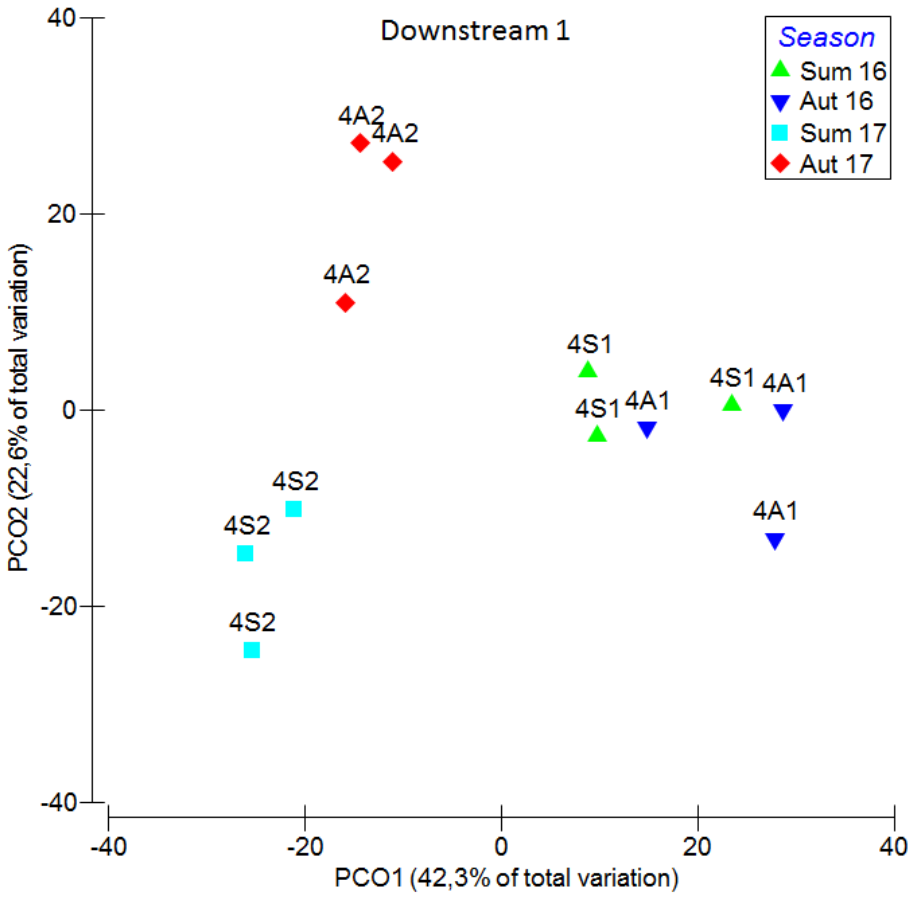

Upstream 1
Season
Sum 16
Aut 16
Sum 17
Aut 17
Similarity
60
70
80
90
100
1S1
1S1
1A1
1A1
1S1
1A1
1S2
1S2
1S2
1A2
1A2
1A2

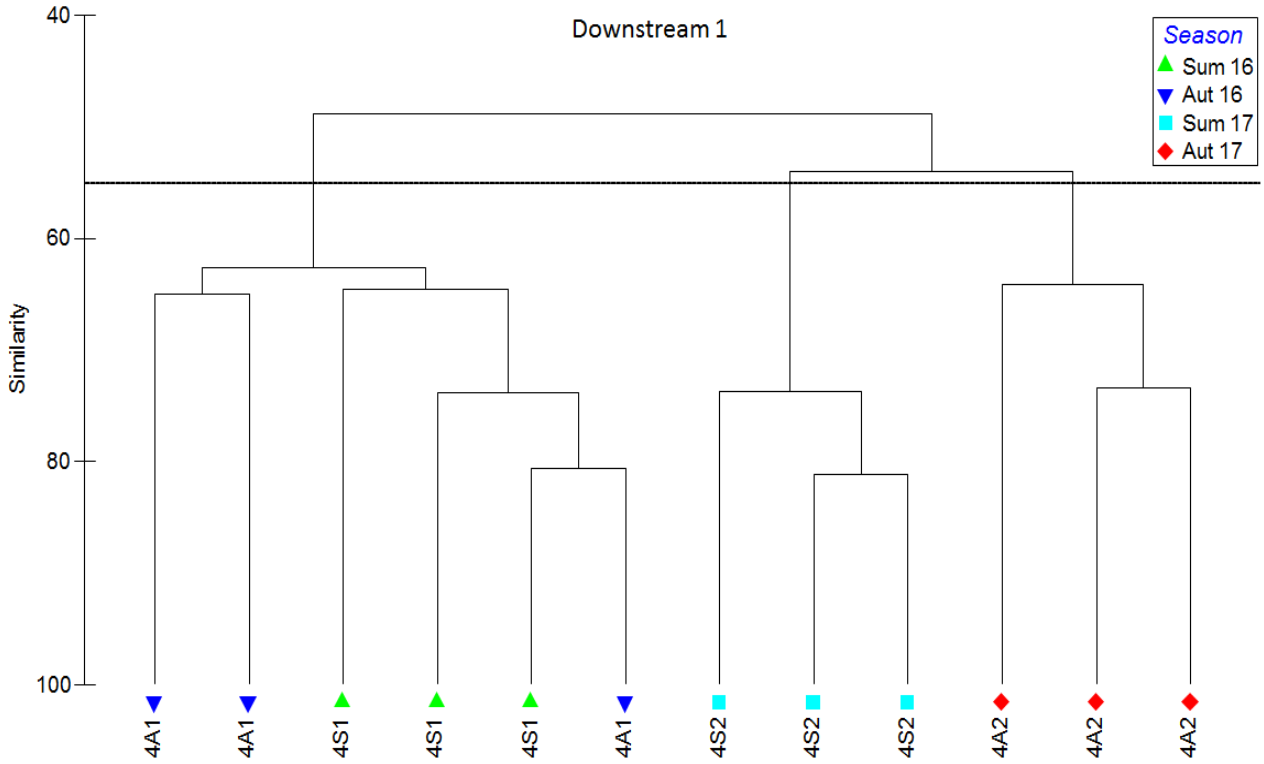

Upstream 2
Season
Sum 16
Aut 16
Sum 17
Aut 17
PCO2 (16,4% of total variation)
PCO1 (55,2% of total variation)
40
20
0
-20
-40
2A1
2A2
2S1
2S2

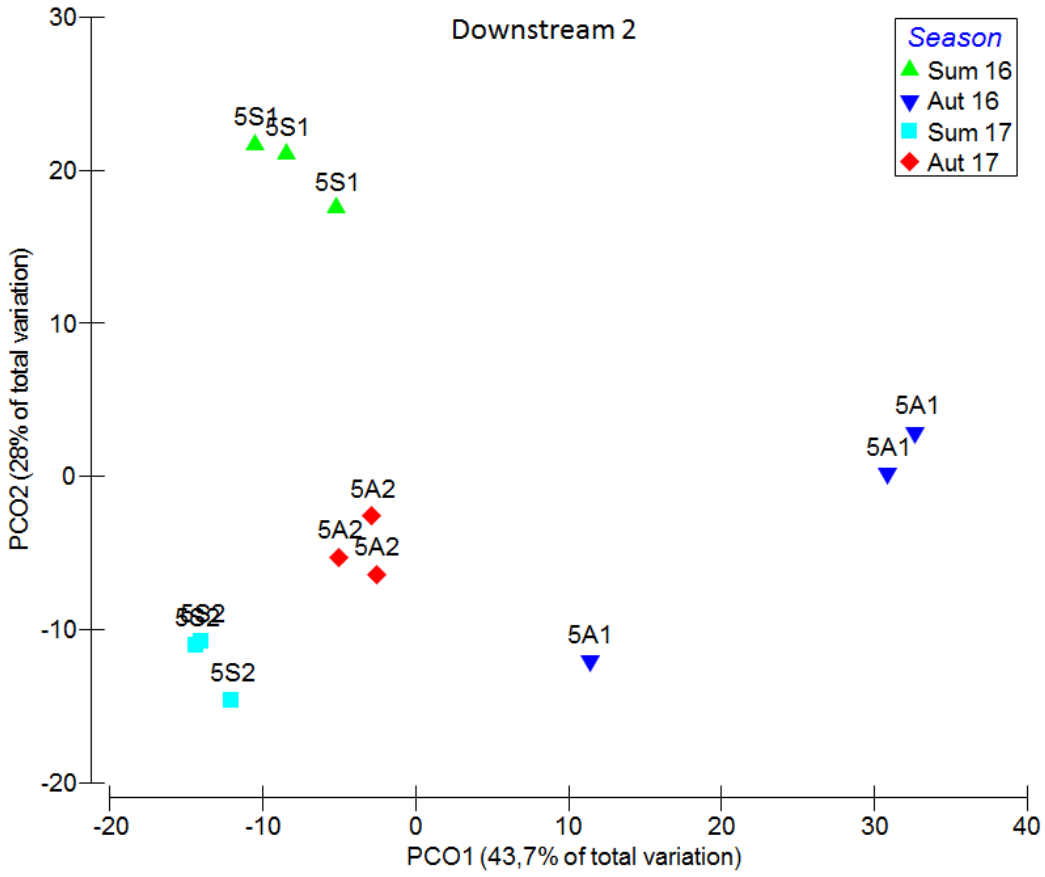

Upstream 2
Season
Sum 16
Aut 16
Sum 17
Aut 17
Similarity
100
80
60
40
2A1
2A2
2S2
2S1

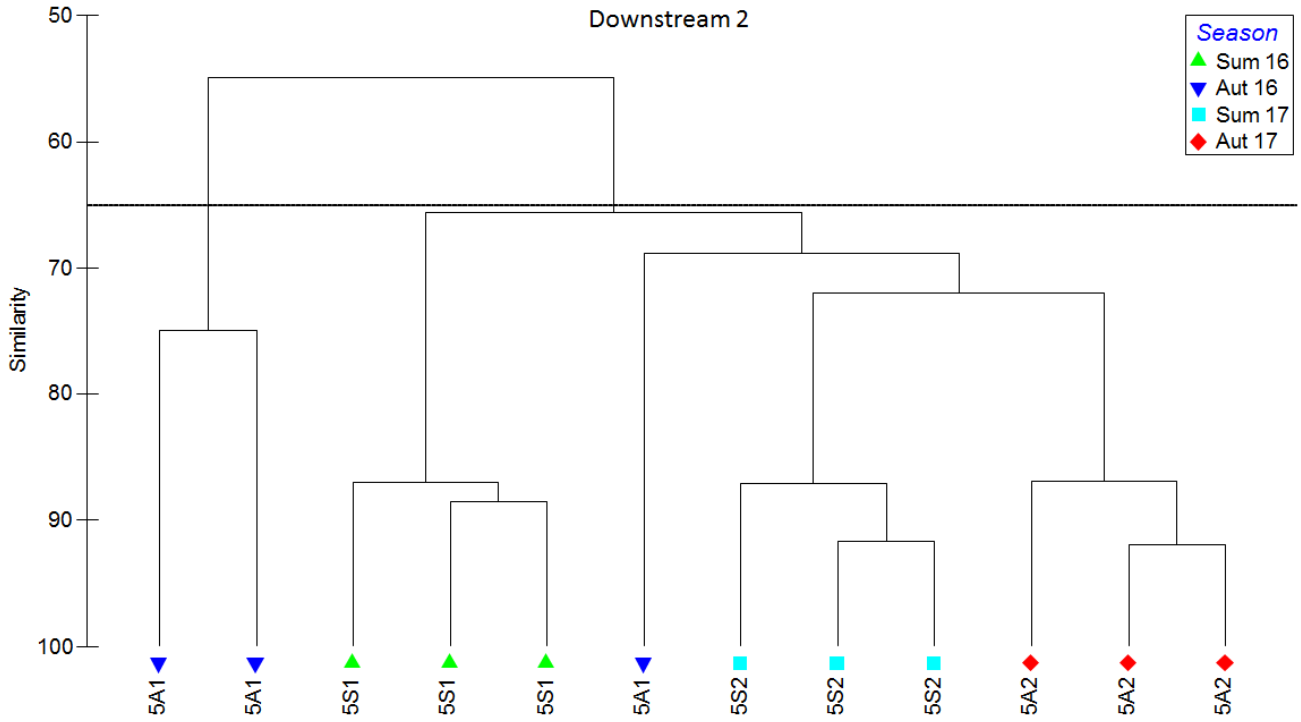

Outlet
Season
Sum 16
Aut 16
Sum 17
Aut 17
3S2
3S1
3A1
3A2
PCO2 (14,1% of total variation)
PCO1 (57,9% of total variation)
20
0
-20
-40
-20
0
20
40

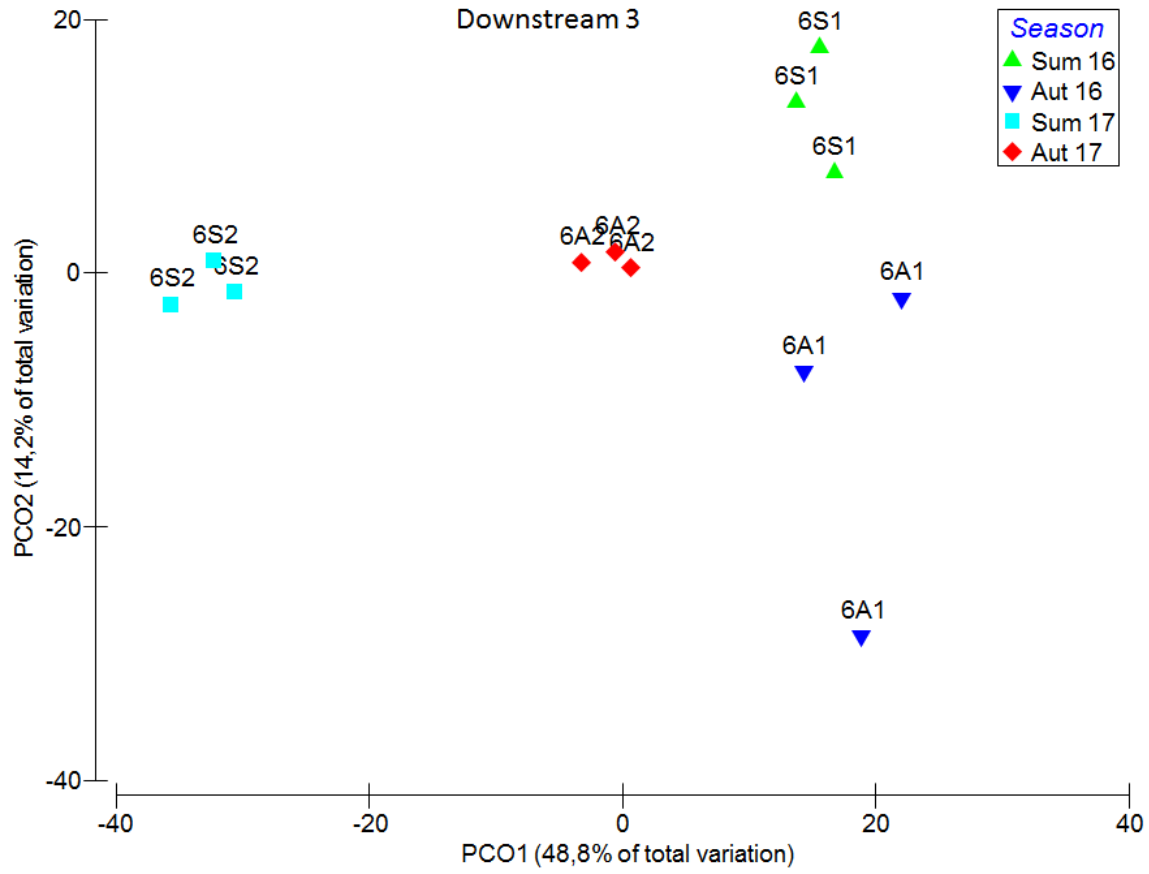

Outlet
Season
Sum 16
Aut 16
Sum 17
Aut 17
Similarity
50
60
70
80
90
100
3S2
3S2
3S2
3A2
3A2
3A2
3S1
3S1
3S1
3A1
3A1
3A1

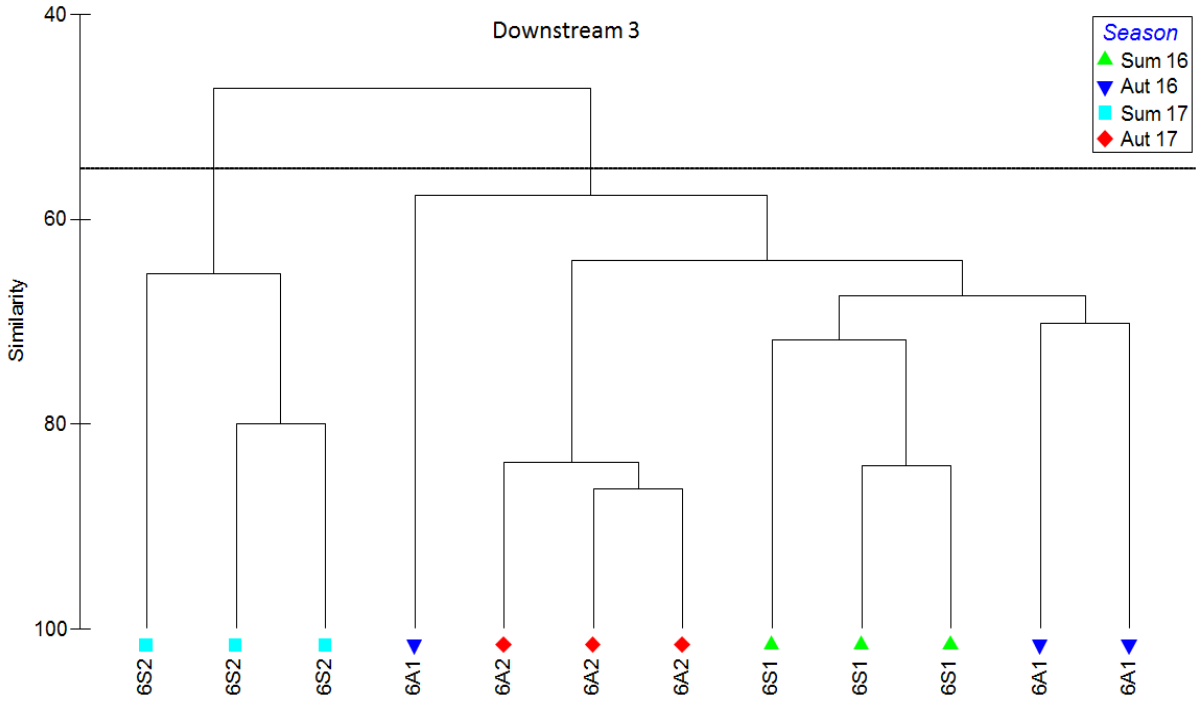

Downstream 1
Season
Sum 16
Aut 16
Sum 17
Aut 17
PCO2 (22,6% of total variation)
PCO1 (42,3% of total variation)
40
20
0
-20
-40
4A2
4S1
4A1
4S2


Downstream 1
Season
Sum 16
Aut 16
Sum 17
Aut 17
Similarity
100
80
60
40
4A1
4S1
4S2
4A2


Downstream 2
Season
Sum 16
Aut 16
Sum 17
Aut 17
PCO2 (28% of total variation)
PCO1 (43,7% of total variation)
30
20
10
0
-10
-20
-20
-10
0
10
20
30
40
5S1
5S1
5S1
5A1
5A1
5A2
5A2
5A2
5S2
5S2
5A1
5S2


Downstream 2
Season
Sum 16
Aut 16
Sum 17
Aut 17
Similarity
50
60
70
80
90
100
5A1
5A1
5S1
5S1
5S1
5A1
5S2
5S2
5S2
5A2
5A2
5A2


Downstream 3
Season
Sum 16
Aut 16
Sum 17
Aut 17
6S1
6S1
6S1
6S2
6S2
6S2
6A2
6A2
6A2
6A1
6A1
6A1
20
0
-20
-40
-40
-20
0
20
40
PCO2 (14,2% of total variation)
PCO1 (48,8% of total variation)


Downstream 3
Season
Sum 16
Aut 16
Sum 17
Aut 17
40
60
80
100
Similarity
6S2
6S2
6S2
6A1
6A2
6A2
6A2
6S1
6S1
6S1
6A1
6A1

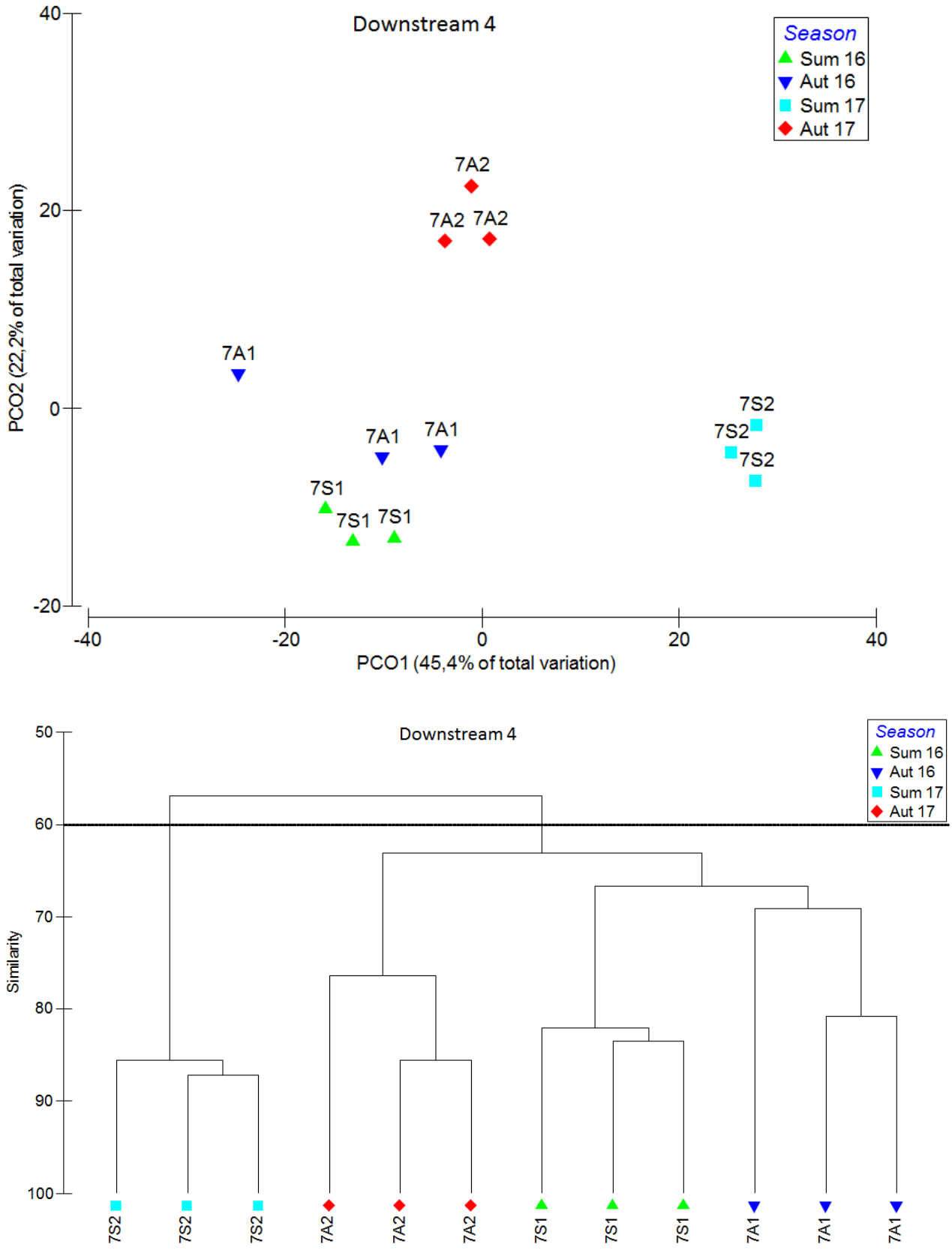


Figure 4.20: Two dimensional PCO plot and Cluster analysis for macroinvertebrates fauna of each station over four sampling times (S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017). Station and season abbreviations as in Figure 4.15

4.3.2.2 SIGNAL 2 index

There were similarities in site scores for the two SIGNAL methods (with and without weighting factor) for most of stations over time. The water quality ratings were different between upstream and downstream over time (Table 4.7). Although the two upstream stations were mostly rated *healthy habitat* for both seasons in both years, site score of those two stations in 2017 were higher than in 2016 (Table 4.7) which indicates better stream conditions after the flood in winter 2016.

The outlet station was rated *mild pollution* at each time. However, the site score fluctuated over the time and was 5.20 and 5.92 in summer 2016; and 5.35 and 5.40 in autumn 2016 (without and with weighting factor). Site score in summer 2017 then increased slightly to 5.71 and 5.69 for without and with weighting factor respectively; suggesting a reduction in level of impact. There was a slight decrease in site score in autumn 2017 (Table 4.7).

Within the four downstream stations, site scores were lowest in autumn 2017, indicating *moderate pollution* at downstream 1, 2 and 3; but *mild pollution* at downstream 4. In general, site score was higher in summer 2016 at each station before decreasing in autumn 2016. Higher site scores occurred at each station in summer and autumn 2017, suggesting the flood positively influenced stream macroinvertebrates. Furthermore, the further downstream station (downstream 4) had a higher site score and better water quality rating compared to the other three downstream stations. Table 4.7 suggests that the level of pollution (from most to least impacted), was outlet (3) and the next three downstream stations (4, 5 and 6), downstream 4 (7). The two upstream stations (1 and 2) appeared to be clean stations.

Table 4.7: Water quality rating at 7 stations in summer and autumn in 2016 and 2017 based on SIGNAL 2 scores calculated with and without an abundance weighting factor

Station	Season	Site score	Water quality rating	Site score (weighting factor)	Water quality rating (weighting factor)
Upstream 1 (1)	Sum 16	6.28	Healthy habitat	6.04	Healthy habitat
Upstream 2 (2)	Sum 16	6.03	Healthy habitat	5.92	Mild pollution
Outlet (3)	Sum 16	5.20	Mild pollution	5.44	Mild pollution
Downstream 1 (4)	Sum 16	4.88	Moderate pollution	5.03	Mild pollution
Downstream 2 (5)	Sum 16	5.56	Mild pollution	5.59	Mild pollution
Downstream 3 (6)	Sum 16	5.07	Mild pollution	5.23	Mild pollution
Downstream 4 (7)	Sum 16	5.43	Mild pollution	5.64	Mild pollution
Upstream 1 (1)	Aut 16	6.61	Healthy habitat	6.07	Healthy habitat
Upstream 2 (2)	Aut 16	6.02	Healthy habitat	5.81	Mild pollution
Outlet (3)	Aut 16	5.35	Mild pollution	5.40	Mild pollution
Downstream 1 (4)	Aut 16	4.97	Moderate pollution	5.33	Mild pollution
Downstream 2 (5)	Aut 16	4.92	Moderate pollution	5.57	Mild pollution
Downstream 3 (6)	Aut 16	4.88	Moderate pollution	4.94	Moderate pollution
Downstream 4 (7)	Aut 16	5.25	Mild pollution	5.25	Mild pollution
Upstream 1 (1)	Sum 17	6.44	Healthy habitat	6.20	Healthy habitat
Upstream 2 (2)	Sum 17	6.68	Healthy habitat	6.17	Healthy habitat
Outlet (3)	Sum 17	5.71	Mild pollution	5.69	Mild pollution
Downstream 1 (4)	Sum 17	5.97	Mild pollution	5.62	Mild pollution
Downstream 2 (5)	Sum 17	5.77	Mild pollution	5.57	Mild pollution
Downstream 3 (6)	Sum 17	5.61	Mild pollution	5.50	Mild pollution
Downstream 4 (7)	Sum 17	5.95	Mild pollution	5.74	Mild pollution

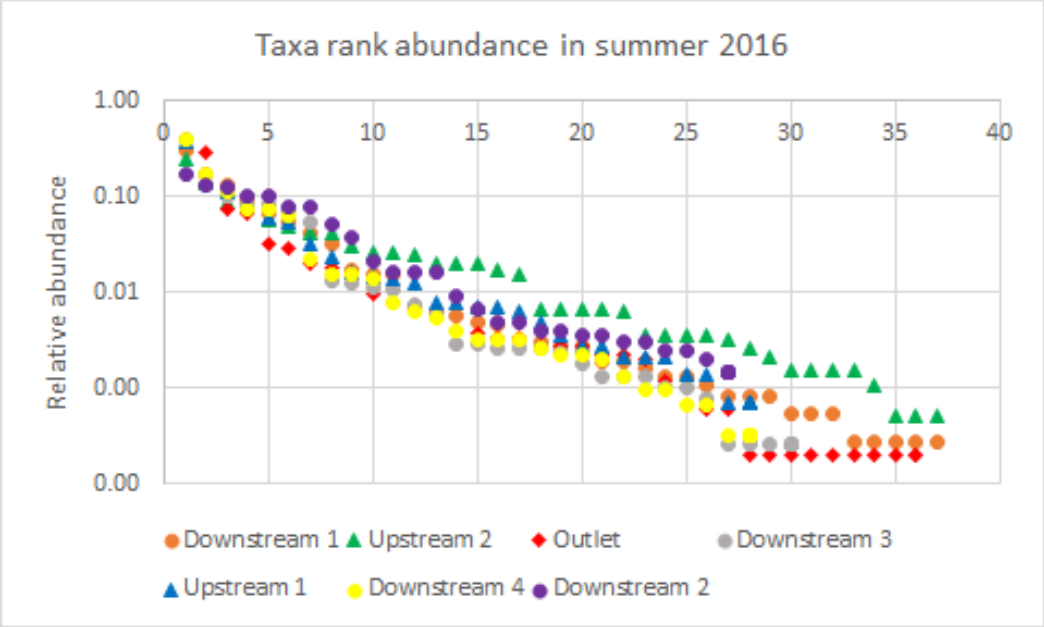
Upstream 1 (1)	Aut 17	6.23	Healthy habitat	5.77	Mild pollution
Upstream 2 (2)	Aut 17	6.71	Healthy habitat	6.08	Healthy habitat
Outlet (3)	Aut 17	5.12	Mild pollution	5.43	Mild pollution
Downstream 1 (4)	Aut 17	5.52	Mild pollution	5.53	Mild pollution
Downstream 2 (5)	Aut 17	5.45	Mild pollution	5.30	Mild pollution
Downstream 3 (6)	Aut 17	5.53	Mild pollution	5.40	Mild pollution
Downstream 4 (7)	Aut 17	5.63	Mild pollution	5.63	Mild pollution

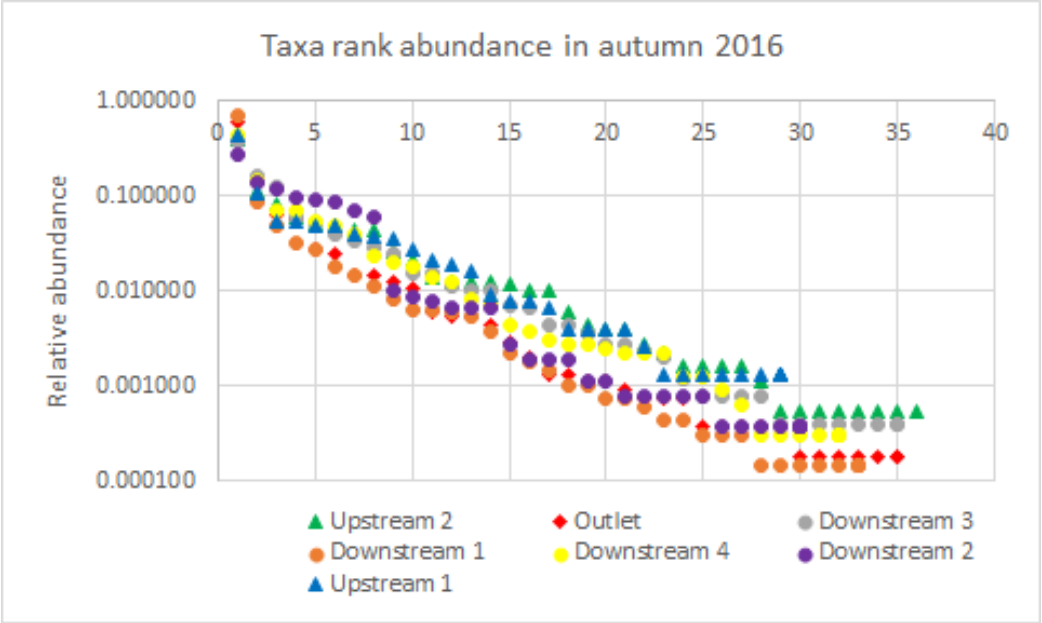
4.3.2.3 Relative abundance

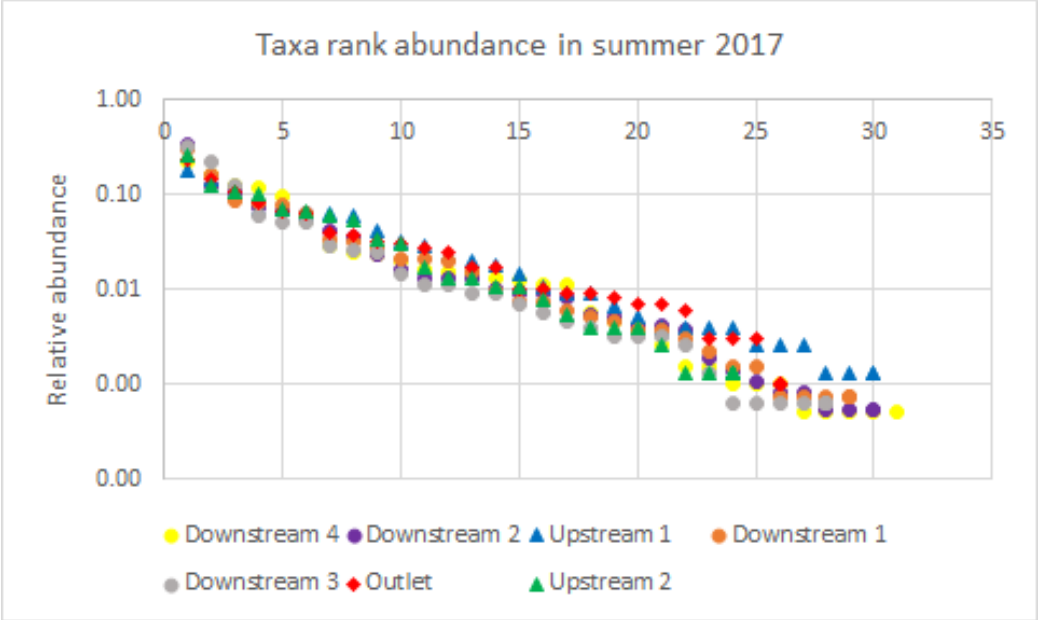
Rank-abundance plots illustrated flatter dominance-diversity curves for all stations in 2017 than in 2016 (particularly in autumn 2017); which suggests the communities after the flood were richer in taxon diversity and had a more even distribution of abundance (Figure 4.21). The flatter curves at all stations in 2017 indicated their relative abundance of taxa in community was similar in summer and autumn 2017. In contrast, steeper dominance-diversity curves were seen for downstream 1 and the outlet stations in autumn 2016; which indicated a lower diversity and a high number of dominant species.

The highest relative abundance for any taxa occurred in autumn 2016: 0.71 at downstream 1 and 0.60 at the outlet (Oligochaeta at both stations) with the second most abundant taxon being 0.08 and 0.14 respectively (Table 4.8). In contrast, the highest relative abundance at other stations was more even and ranged from 0.27 to 0.43 with the second most abundant taxon relatively similar (Figure 4.21, Table 4.8). In summer 2016, the highest relative abundance (0.40) were seen at downstream 3, followed by outlet (0.39), downstream 4 (0.39), upstream 1 (0.37), downstream 1 (0.31), upstream 2 (0.25) and upstream 1 (0.17). In summer 2017, the highest relative abundance ranged from 0.18 (at upstream 1) to 0.34 (at

downstream 2) while that was from 0.20 (at outlet) to 0.39 (at upstream 2) in autumn 2017 (Figure 4.21, Table 4.8).







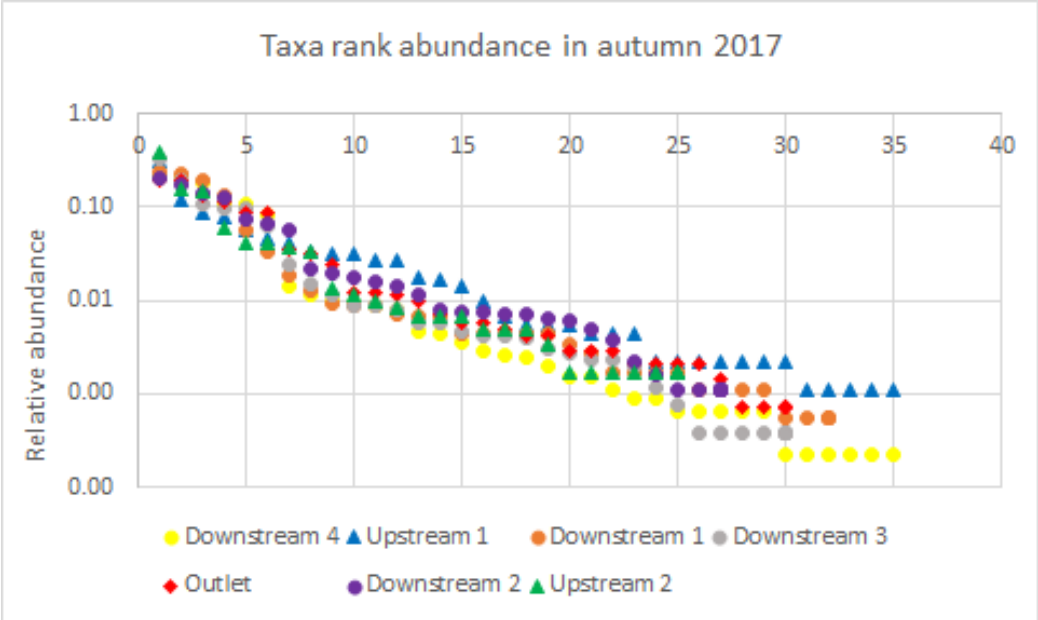
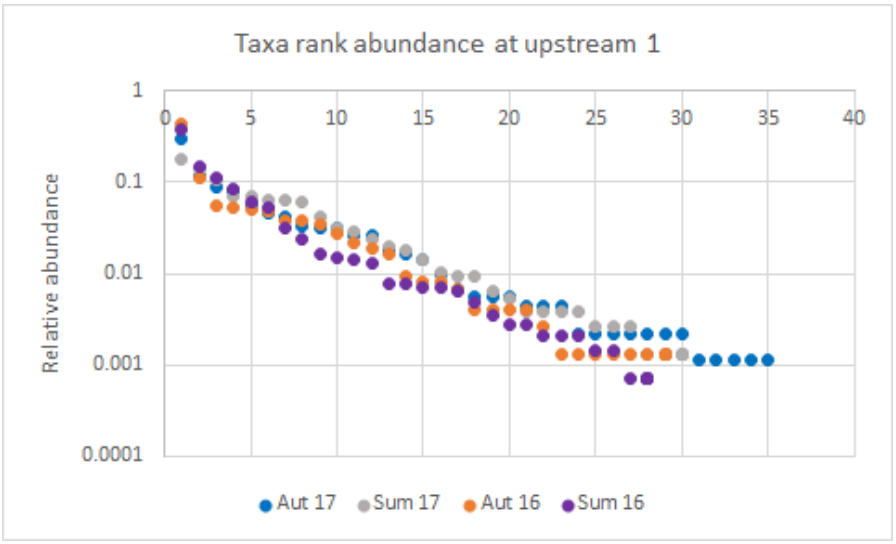
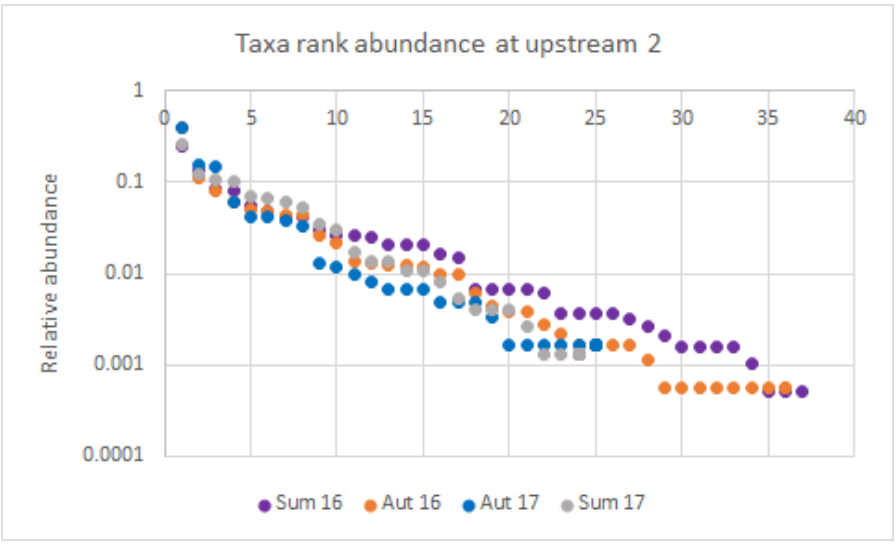
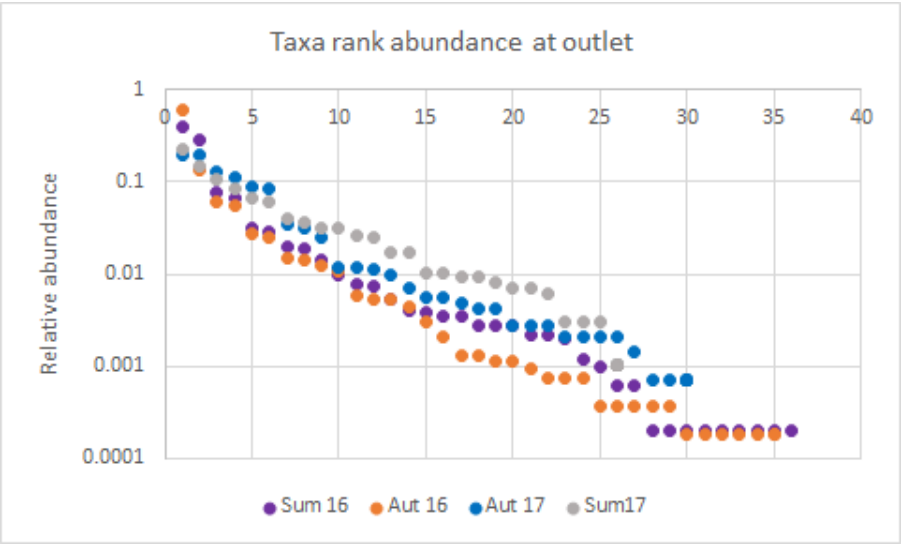
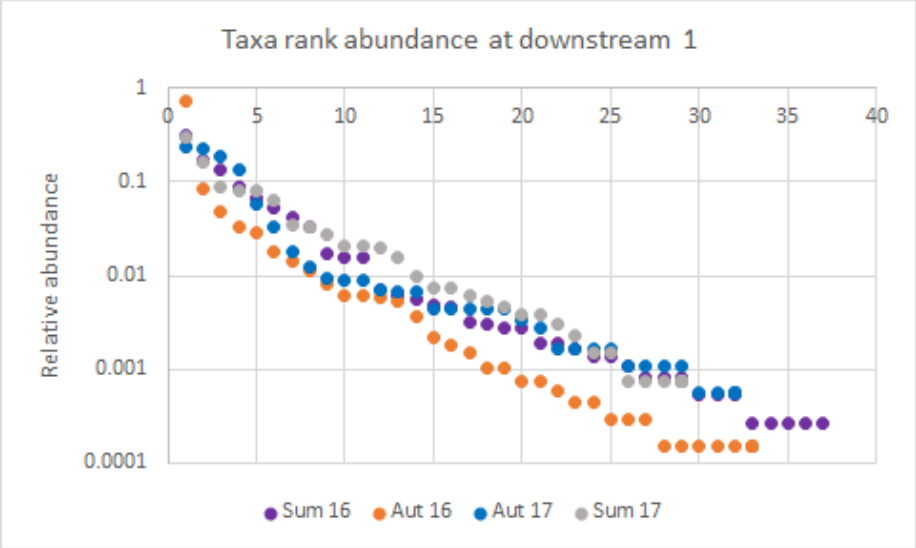


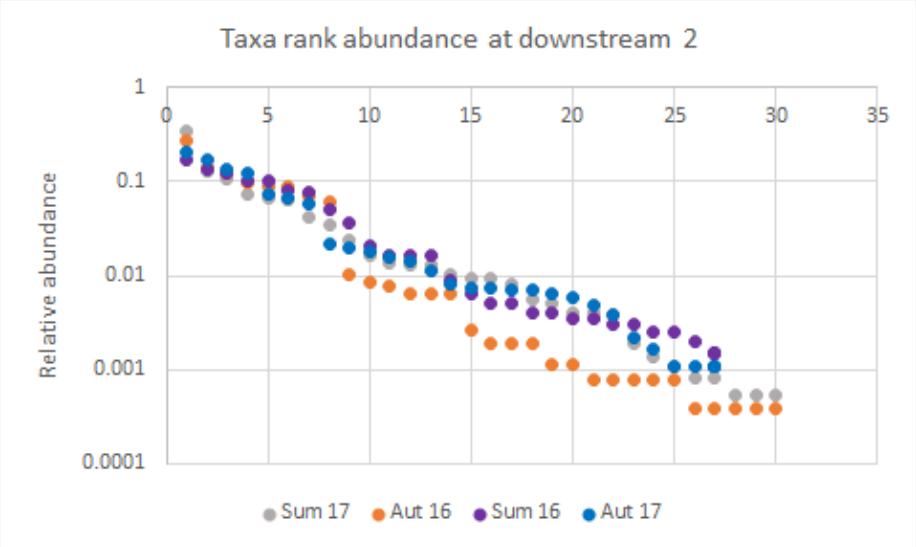
Figure 4.21: The dominance-diversity distribution for 7 Florentine River stations in two seasons (summer, autumn) over two years (2016, 2017)

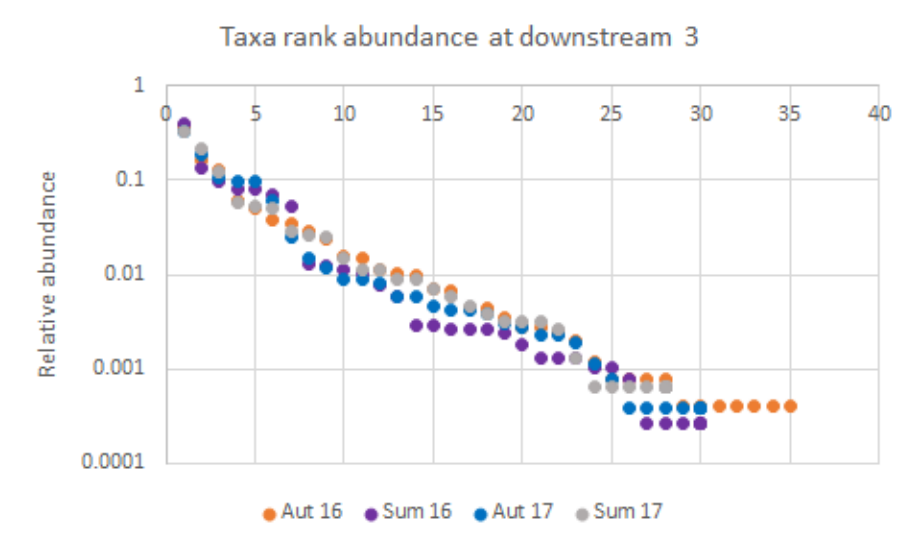

Taxa rank abundance at upstream 1
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Aut 17
Sum 17
Aut 16
Sum 16


Taxa rank abundance at upstream 2
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Sum 16
Aut 16
Aut 17
Sum 17


Taxa rank abundance at outlet
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Sum 16
Aut 16
Aut 17
Sum17


Taxa rank abundance at downstream 1
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Sum 16
Aut 16
Aut 17
Sum 17


Taxa rank abundance at downstream 2
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
Sum 17
Aut 16
Sum 16
Aut 17


Taxa rank abundance at downstream 3
Relative abundance
1
0.1
0.01
0.001
0.0001
0
5
10
15
20
25
30
35
40
Aut 16
Sum 16
Aut 17
Sum 17

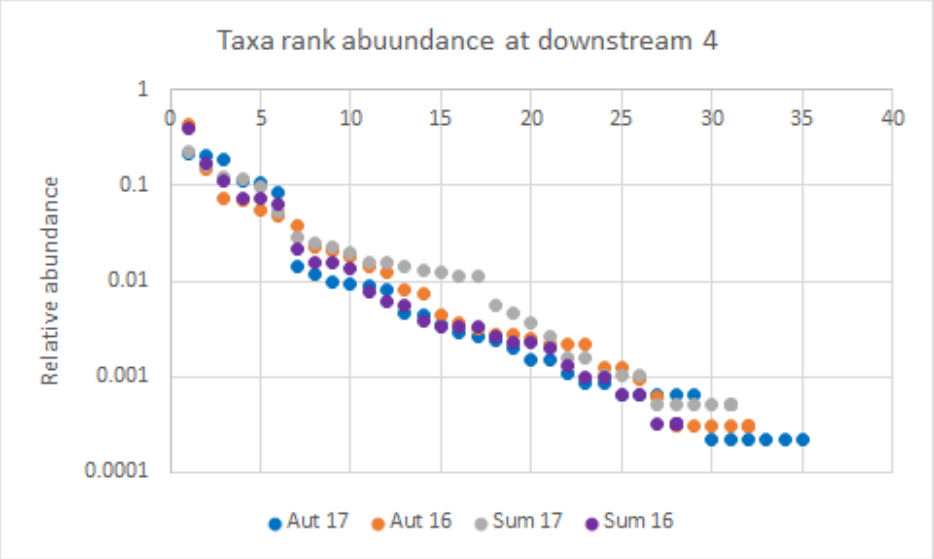


Figure 4.22: The dominance-diversity distribution for 4 sampling times (summer 2016, autumn 2016, summer 2017, autumn 2017) at each station

Table 4.8: The three most dominant taxa and their relative abundance at seven stations (pooled across time). Station and season abbreviations as in Figure 4.15

Station/Season	Total N. of taxa	First 3 dominated taxa	Relative abundance	Individuals of each taxa/ total individuals
1S1	28	Baetidae	0.37	525/1421
		Leptophlebiidae	0.15	210/1421
		Costora delora	0.11	157/1421
1A1	29	Baetidae	0.43	323/750
		Costora delora	0.11	82/750
		Leptophlebiidae	0.05	41/750
1S2	30	Conoesucidae	0.18	137/767
		Baetidae	0.14	110/767
		Costora delora	0.11	83/767
1A2	35	Baetidae	0.30	274/901
		Costora delora	0.12	106/901
		Hydropsychidae	0.09	80/901
2S1	37	Baetidae	0.25	473/1917
		Leptophlebiidae	0.14	259/1917
		Elmidae (L)	0.09	163/1917
2A1	36	Baetidae	0.39	711/1804
		Scirtidae	0.11	206/1804
		Lingora sp.	0.08	145/1804
2S2	24	Baetidae	0.25	192/753
		Conoesucidae	0.12	92/753
		Elmidae (A)	0.11	82/753
2A2	25	Baetidae	0.39	239/606
		Gripopterygidae	0.15	93/606
		Lingora sp.	0.15	89/606
3S1	36	Oligochaeta	0.39	1943/4975
		Physa acuta	0.29	1436/4975
		Orthocladiinae	0.08	374/4975
3A1	35	Oligochaeta	0.60	3263/5400
		Planorbidae	0.14	734/5400
		Physa acuta	0.06	333/5400

3S2	26	Oligochaeta	0.23	223/974
		Tanypodinae	0.15	144/974
		Conoesucidae	0.11	106/974
3A2	30	Baetidae	0.20	281/1418
		Oligochaeta	0.19	275/1418
		Tanypodinae	0.13	185/1418
4S1	37	Oligochaeta	0.31	1134/3702
		Planorbidae	0.17	641/3702
		Orthocladiinae	0.13	498/3702
4A1	33	Oligochaeta	0.71	4800/6724
		Planorbidae	0.08	570/6724
		Orthocladiinae	0.05	325/6724
4S2	29	Baetidae	0.30	398/1328
		Leptophlebiidae	0.16	212/1328
		Costora delora	0.09	116/1328
4A2	32	Chironominae	0.24	429/1819
		Orthocladiinae	0.23	418/1819
		Baetidae	0.19	345/1819
5S1	27	Orthocladiinae	0.17	348/1998
		Tanypodinae	0.13	265/1998
		Leptophlebiidae	0.12	243/1998
5A1	30	Orthocladiinae	0.27	704/2604
		Baetidae	0.14	364/2604
		Hydropsychidae	0.12	315/2604
5S2	30	Baetidae	0.34	1237/3666
		Leptophlebiidae	0.13	463/3666
		Orthocladiinae	0.11	396/3666
5A2	27	Chironominae	0.21	386/1860
		Baetidae	0.17	318/1860
		Orthocladiinae	0.14	254/1860
6S1	30	Hydropsychidae	0.40	1517/3837
		Chironominae	0.13	516/3837
		Orthocladiinae	0.10	380/3837
6A1	35	Hydropsychidae	0.37	934/2524
		Planorbidae	0.16	403/2524

		Orthocladiinae	0.13	321/2524
6S2	28	Baetidae	0.32	496/1541
		Leptophlebiidae	0.22	335/1541
		Leptoceridae	0.13	193/1541
6A2	30	Chironominae	0.33	844/2591
		Orthocladiinae	0.19	492/2591
		Hydropsychidae	0.11	276/2591
7S1	28	Hydropsychidae	0.39	1203/3066
		Chironominae	0.17	534/3066
		Baetidae	0.11	339/3066
7A1	32	Hydropsychidae	0.43	1396/3215
		Orthocladiinae	0.15	474/3215
		Baetidae	0.07	231/3215
7S2	31	Baetidae	0.23	442/1943
		Conoesucidae	0.17	322/1943
		Leptophlebiidae	0.12	241/1943
7A2	35	Chironominae	0.22	990/4597
		Orthocladiinae	0.20	936/4597
		Baetidae	0.19	863/4597

The three most dominant taxa and their relative abundance indicated the dominant species at the two upstream stations (1 and 2) did not change substantially after the flood but there were differences in the dominant species at the outlet (3) and downstream stations (4, 5, 6 and 7) after the flood (Table 4.8). At the upstream stations (1 and 2), Baetidae was the most dominant taxa over the four sampling times while Leptophlebiidae, Conoesucidae, *Costora Delora* (Conoesucidae), *Lingora sp.* (Conoesucidae) and Gripopterygidae were also inhabitants at upstream stations. Those species are typically very sensitive to pollution. In contrast, changes in the three most dominant species were seen at the outlet (3) and downstream stations (4, 5, 6 and 7) after the flood. The key species (pollution tolerant species:

Oligochaeta, Planorbidae, *Physa acuta*) were strongly dominant at the outlet (3) and downstream 1 (4) stations in summer and autumn 2016 whereas Baetidae, Leptophlebiidae, Conoesucidae and *Costora delora*, which are pollution intolerant species, were dominant at those stations in summer and autumn 2017 suggesting an improvement in conditions. At the other downstream stations (5, 6 and 7), the most dominant species included both pollution tolerant and intolerant species (Table 4.8), which would indicate that level of impact appeared to be lower than at the outlet (3) and downstream 1 (4) stations. At downstream 2 (5), Orthocladiinae was present in high numbers in 2016 while Baetidae dominated in 2017. Downstream 3 (6) was dominated by Hydropsychidae, Chironominae, Orthocladiinae and Planorbidae in 2016 whilst Baetidae, Leptophlebiidae and Leptoceridae as well as Chironominae, Orthocladiinae and Hydropsychidae were dominant in summer and autumn 2017 respectively. At downstream 4 (7), Hydropsychidae, Chironominae, Orthocladiinae and Baetidae dominated in 2016 whereas Baetidae, Conoesucidae and Leptophlebiidae as well as Chironominae, Orthocladiinae and Baetidae were the most three dominant species in summer and autumn 2017 respectively (Table 4.8). Generally, the results showed pollution intolerant species were abundant at stations (5, 6 and 7) further downstream compared to outlet (3) and downstream 1 (4) stations as well as being higher in 2017 compared to 2016. This suggests a recovery progress from downstream 2 station and further downstream with less impact on macroinvertebrate communities after the flood at these stations. Furthermore, this also suggests a gradient of communities from very pollution-sensitive to very pollution-tolerant taxa from upstream 1 (1), upstream 2 (3), downstream 4 (7), downstream 3 (6), downstream2 (5), downstream 1 (4) to the outlet (1).

4.3.2.4 Total abundance, taxa richness, Simpson diversity index

There was a significant interaction between station and season for total abundance, taxa richness and Simpson diversity index of macroinvertebrates (PERMANOVA, $F_{18,56}$=3.73, 2.38, 2.85, P_{MC}=0.0002, 0.0068, 0.001; respectively) (Table 4.9). It was apparent that the effects of different sampling times were dependent on the distance of the station from the outfall. With the exception of summer 2016, a higher total abundance was observed at downstream stations (3 to 7) than at upstream stations (1 and 2) whereas there were no substantial changes in taxa richness between upstream and downstream stations (Figures 4.23, 4.24). Simpson's diversity fluctuated between stations over four sampling times. After the flood, with the exception of upstream 1 (1) and downstream 2 (5), stations experienced a decrease in total abundance and taxa richness (Figure 4.23, 4.24) while higher diversity indices were seen at most stations (Figure 4.25).

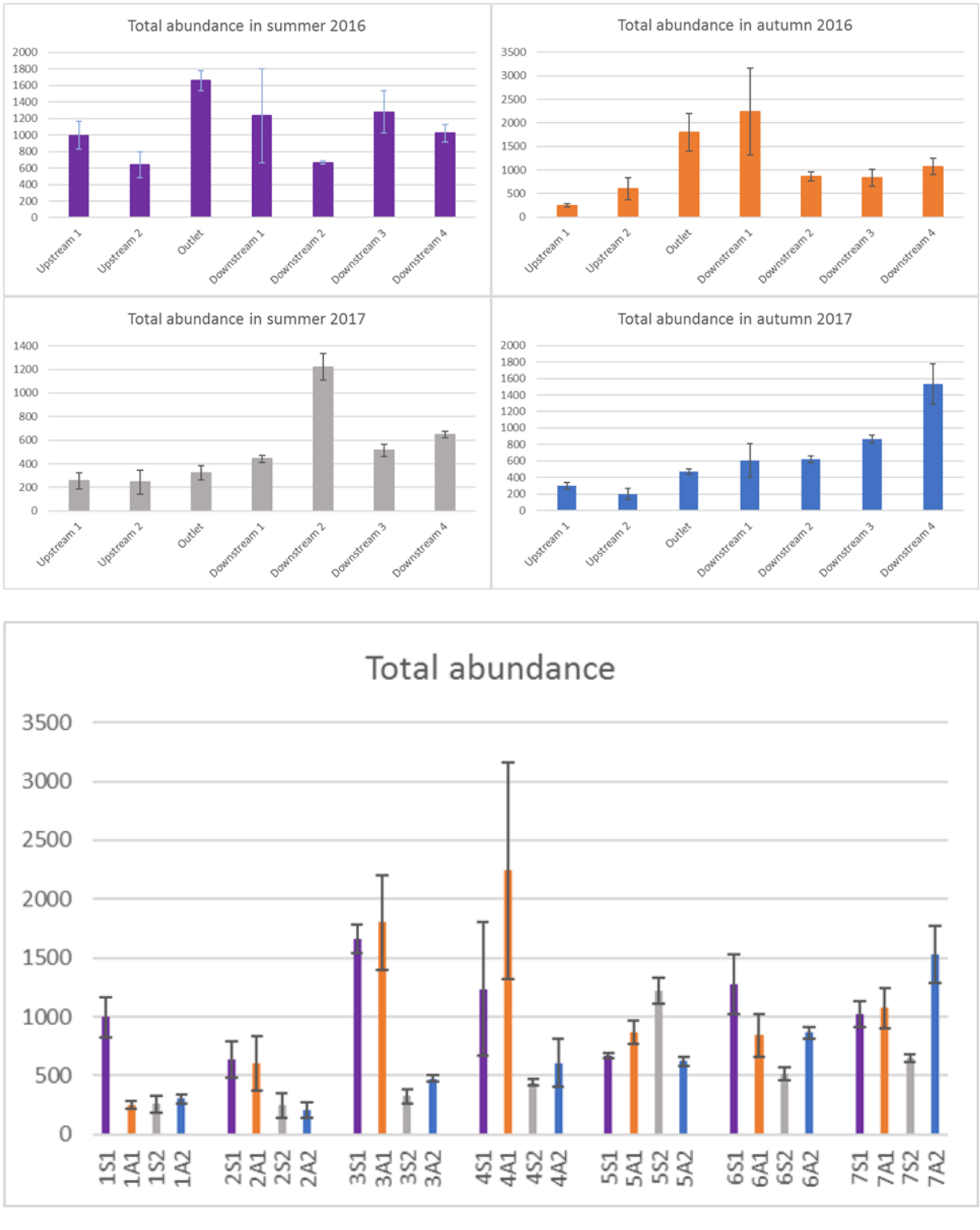


Figure 4.23: Mean (±SE; n=3 replicates) of total abundance of macroinvertebrates in seven stations at Florentine over four sampling times.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

Figure 4.24: Mean (±SE; n=3 replicates) of taxa richness of macroinvertebrates in seven stations at Florentine over four sampling times.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

Figure 4.25: Mean (±SE; n=3 replicates) of Simpson diversity index of macroinvertebrates in seven stations at Florentine over four sampling times.
(Note: the bottom panel does repeat the data in the top panel redrawn to visualise the seasonal changes at each station)

There was a significant station x season interaction for total abundance (Figure 4.23, Table 4.9). In summer 2016, total abundance at the outlet station (3) (1658 individuals) was significantly higher than the two upstream stations (1 and 2) (996 and 639 individuals), downstream 2 (5) (666 individuals) and downstream 4 (7) (1022 individuals) but did not differ significantly from downstream 1 (4) (1234 individuals) and downstream 3 (6) (1279 individuals) (Table 4.9). In autumn 2016, total abundance of upstream 1 (1) was lowest (250 individuals) and significantly different from other stations. In summer 2017, downstream 2 (5) and downstream 4 (7) stations (1222 and 647 respectively) differed significantly from other stations while only downstream 4 (7) had significantly higher abundance (1532 individuals) than other stations in autumn 2017. In contrast, there were no significant differences in total abundance between stations in autumn 2017. Furthermore, the highest total abundance between the four sampling times was seen in autumn 2016 at downstream 1 (4), which was 2241 individuals, followed by the outlet (3) (1800 individuals) whereas lowest total abundance were recorded at the two upstream stations (1 and 2), being 250 individuals at upstream 1 in autumn 2016 and 201 individuals at upstream 2 in autumn 2017 (Figure 4.23).

There was a significant station x season interaction for taxa richness although there were slight differences between stations over four sampling times. In summer 2016, taxa richness between stations ranged from 23 to 27 while those numbers were from 20 to 25 taxa in autumn 2016. Taxa richness at upstream 1 (1) differed significantly from only upstream 2 in summer 2016 whereas that of downstream 2 (5) was significantly different from outlet (3) and downstream 4 (7) in autumn 2016 (Figure 4.24, Table 4.9). In summer 2017, taxa richness

at each station was mostly lower than autumn 2016, ranging from 19 to 27 taxa. Downstream 2 (5) showed highest taxa richness in autumn 2016 and was significantly higher than upstream 2 (2), downstream 1 (4) and downstream 3 (6). Taxa richness in autumn 2017 ranged between 15 and 27; and taxa richness of upstream 2 (2) were significantly higher than other station except for downstream 1 (4). Overall, there were no significant differences in taxa richness between times at each station (Table 4.9); except upstream 2 (2) and downstream 2 (5). At upstream 2 (2), there was significantly higher taxa richness between summer 2016, and summer 2017 and autumn 2017. At downstream 2, taxa richness in autumn 2016 differed significantly from summer 2016 and 2017 (Figure 4.24).

There was a significant station x season interaction for Simpson diversity index (Figure 2.25, Table 4.9). Lower diversity indices were seen at the outlet (3) and downstream 1 (4) stations than other stations in summer and autumn 2016. In 2016, downstream 2 (5) also had the highest taxa richness compared to other stations: 9.92 in summer 2016 and 6.14 in autumn 2016. In summer 2016, downstream 2 (5) had a significantly higher taxa richness than upstream stations (1 and 2), outlet (3) and other downstream stations (4, 6 and 7) while that of the outlet (3) was significantly lower that upstream 2 (2) and downstream 1 (4). In autumn, diversity of downstream 2 (5) was significantly higher than that of outlet (3) and downstream 1 (4). In summer 2017, the diversity index increased at all stations ranging between 5.82 and 10.56 while those in autumn 2017 ranged from 4.72 to 8.3. There was a significantly lower diversity index between downstream 2 (5) and upstream 1 (1) in summer 2017 whereas diversity of downstream 2 significantly differed from upstream 2 (2), downstream 1 (4) and further downstream station (7 and 8). Generally, there were no clear differences between all downstream stations over the four sampling times, except summer 2016. Diversity index at

each station over four sampling times showed that similar diversity index between summer and autumn 2016 was seen at all stations, except downstream 1 (4) and downstream 2 (5) while diversity between summer and autumn 2017 was similar at all stations even though there were no significant differences in diversity index between four sampling times at downstream 3 and 4 (6 and 7).

Table 4.9: ANOVA testing Station (St), Season (Se) and Station x Season (StxSe) on total abundance, taxa richness and Simpson diversity index of macroinvertebrate community. Analyses were based on Euclidean distance with data transformed in square root. Permutations (N=9,999) were applied to the residuals under a reduced mode. Pair-wise post hoc comparisons were done for station, season and station x season

Source	Df	P_{seudo}-F	P (MC)	Post hoc comparison	P_{seudo}-F	P (MC)	Post hoc comparison
Transformation		Total abundance Square root			Taxa richness Square root		
St	6	12.684	0.0001		1.1862	0.3228	
Se	3	13.034	0.0001		2.106	0.1088	
StxSe	18	3.7344	0.0002	5S1≠7S1	2.3803	0.0068	
				3S1≠1S1,2S1,5S1,7S1			
				1A1≠3A1,4A1,5A1,6A1,7A1			1S1≠2S1 5A1≠3A1,7A1 5S2≠2S2,4S2,6S2 2A2≠1A2,3A2,5A2,6A2,7A2 4A2≠7A2
				5S2≠1S2,2S2,3S2,4S2,6S2,7S2			
				7S2≠1S2,2S2,3S2,4S2,5S2			
				1S2≠6S2			
				1A2≠3A2,5A2,6A2,7A2			1S1=1A1=1S2=1A2 2S1#2S2,2A2 3S1 = 3A1 = 3S2 = 3A2 4S1 = 4A1 = 4S2 = 4A2 5A1#5S1,5S2 6S1 = 6A1 = 6S2 = 6A2 7S1 = 7A1 = 7S2 = 7A2
				2A2≠3A2,5A2,6A2,7A2			
				3A2≠5A2,6A2,7A2			
				7A2≠4A2,5A2,6A2			
				5A2≠6A2			
				1S1=1A1=1S2=1A2			
				2S1=2A1 = 2S2 = 2A2			
				3S1 = 3A1 = 3S2 = 3A2			
				4S1≠4S2			
				5S2≠5S1, 5A2			
				6S2≠6S1, 6A2			
				7S2≠7S1, 7A2			
Residuals	56						
Transformation		Simpson diversity index					

		Square root		
St	6	4.3081	0.0009	
Se	3	18.853	0.0001	
StxSe	18	2.8505	0.001	1S1≠2S1,5S1 2S1≠1S1,3S1,5S1,6S1 3S1≠2S1,4S1,5S1 4S1≠3S1,5S1 5S1≠1S1,2S1,3S1,4S1,6S1,7S1 5A1≠3A1,4A1 1S2≠5S2 5A2≠2A2,4A2,6A2,7A2 1S1=1A1,1A2; 1A2=1A1,1S2 2S1=2A1,2S2; 2A1=2S2,2A2 3S1=3A1,3S2; 3S2=3A2 4S1=4S2,4A2; 4S2=4A2 5A1=5S2; 5A2=5S1,5S2 6S1=6A1=6S2=6A2 6S1=6A1=6S2=6A2
Residuals	56			

1: upstream1, 2: upstream2, 3: outlet, 4: downstream1, 5: downstream2, 6: downstream3, 7: downstream 4
S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017

4.4 Discussion

This study has shown large variability in the river macroinvertebrate communities among stations at different distances from outfalls of aquaculture hatcheries in two rivers in Tasmania. These findings suggest biological responses can infer stream water quality at this scale which has implications for monitoring and regulatory purposes. Clear changes in macroinvertebrate structure between upstream and downstream were seen at each river. Macroinvertebrate community composition was previously shown to be different between non-farm reference stations in the Florentine River and the Brumbys Ck (Chapter 2 and 3) which likely reflected stream type, geomorphology and location (Crunkilton and Duchrow, 1991). The results from our previous and current chapters indicated the water quality rating upstream of Florentine was better than that of Brumbys; classified as *healthy habitat* and *mild to moderate pollution*, respectively. This might be because of different catchment habitat between the two rivers. The Florentine is a highland river surrounded by native and plantation

forested areas while Brumbys is a lowland river surrounded by agricultural activities. As a result, macroinvertebrate communities at those two rivers might respond differently to waste discharge from the farm. Furthermore, the different responses of macroinvertebrates to discharge effluents might also depend on the production level of the fish farms (Camargo, 2019; Metzeling, 1999) and effluent volume (Metzeling, 1999). Generally, downstream community assemblages were different from those upstream and at the outlet; indicating there were potential impacts of farms on macroinvertebrate community structure. Previous studies have illustrated farming production occurring in the catchment can influence reflected in reduced water quality and changed invertebrate communities (Tello et al., 2010; Webb, 2012b). This is usually because of an increasing volume of nitrogen and phosphorus (Camargo and Gonzalo, 2007; Carr and Goulder, 1990b; Kelly, 1993; Taseli, 2009) and suspended solids (Guilpart et al., 2012; Taseli, 2009) from farm effluents deteriorating stream water quality and biology at downstream sites.

Here, the level of impact generally declined moving further downstream; which is similar to the findings of Camargo (2019) who determined there was a decreasing level of water pollution with increasing distance downstream from the farm discharge point. Loch et al. (1996a) explained that the highest concentration occurred at the outfall, and downstream the river dilutes the effluent depending on the relative size of the river to effluent outfall and the flow of the river.

SIGNAL 2 scores and diversity indices suggest that the Florentine had a higher water quality rating and diversity compared to Brumbys downstream of the outfall even though downstream stations of both rivers were influenced by farm effluents. Furthermore, the macroinvertebrate communities at the outlet and downstream 1 stations at the two rivers

were the most different from upstream and other downstream stations; similar to the findings of Camargo et al. (2011). Research on trout farms also found that the most impact on stream macroinvertebrates was seen from just below the farm outfall to 350 – 500 meters downstream (Camargo, 1994; Webb, 2012b). Interestingly, downstream 1 at Brumbys which was only 150 metres from the outfall, appeared to be less polluted than the outlet whilst downstream 1 at Florentine which was 200 metres away, was more polluted than the outlet. This might be a result of differences in stream conditions. The Brumbys has very slow flow and currents which might cause limited mixing of waste water and matter downstream of the outlet. Therefore, a larger amount of waste might settle near at outlet compared to the downstream from the outlet. In contrast, the Florentine has a very fast and strong flow as well as a high discharge flow at the outlet which may result in a greater movement of waste to downstream 1 and less settlement at the outlet.

Oligochaeta and Hirudinae appeared to be the key indicator taxa for farming impacts at the two streams as the two taxa were dominant at the outfalls Furthermore, those two taxa were present at impacted stations (outfall and closest downstreams) over the four sampling times, their abundance was highest at the outfall, with decreasing abundance moving further away from the outfall. Previous research showed similar findings with Oligochaeta dominant at sites with high organic matter (Armitage et al., 1983a; Camargo, 1994; 2019; Kırkagaç et al., 2004; Rosenberg and Resh, 1993). Moreover, Oligochaeta were also the key taxa differentiating between the upstream and downstream communities with an absence upstream and high abundance at sites immediately downstream of the outfall. Similarly, Brown (2001) detected that aquatic worms were abundant at the farm discharge to approximately 1000 m downstream. In terms of temporal comparisons, invertebrate

composition of each station at the Brumbys did not change much over four seasons and there were only changes in abundance of each taxa. This resulted in slight differences in SIGNAL 2 and biological patterns between seasons at Brumbys. In contrast, at the Florentine, significant differences in community composition occurred between 2016 and 2017 and SIGNAL 2 indicated better water quality rating after the major flood. There was also a decrease in total abundance and taxa richness while diversity of each station increased after the flood. A flood is likely to disturb the habitat, dislodge animals and flush organic matter from the river. The higher diversity index despite a decrease in abundance and richness likely reflects a more even relative abundance of taxa with no dominant species within the community.

Biological metrics (relative abundance, total abundance, taxa richness and Simpson diversity index) did not illustrate the same differences in macroinvertebrate communities between stations as each single metric showed different trending between stations. However, those metrics did demonstrate changes of abundance, richness and diversity of macroinvertebrates between upstream and downstream as well as the relationship between those indices. In particular, stations (outlet and downstream 1) had high total abundance and their relative abundance showed some dominant taxa with high abundance; resulting in low taxa richness and diversity. This was also similar with the findings of the chapter 3 and previous studies which showed that a reduction of taxa richness (Camargo, 1994; Doughty and McPhail, 1995; Guilpart et al., 2012; Lalonde et al., 2016; Selong and Helfrich, 1998) and diversity index at impacted sites (Camargo, 1992a) was correlated with dominance of tolerant taxa.

Overall, results indicated a partial recovery of the macroinvertebrate community occurring at downstream sites; however, macroinvertebrates within 800 m downstream did not return to the same assemblages as the upstream sites in over the four seasons sampling (12 – 18

months). This might suggest the introduction of farm effluents improve the diversity of the invertebrates i.e. enriched the environment enough to make it better; highlighting farm effluents might not always have negative impacts but might also have positive effects on stream invertebrates. This can be a result of good management of farms to minimise nutrients into the receiving water. However, Camargo and Gonzalo (2007) demonstrated that organic pollution and nutrient enrichment from farm activities increased gradually over time. The research of Selong and Helfrich (1998) on impacts of trout farms found that macroinvertebrates partly recovered at 400 m downstream from the farm outfalls. Nevertheless, Loch et al. (1996a) asserted that invertebrates did not fully recover at 1.5 km downstream; which similar to this study showing partial recovery of downstream sites within 800 m downstream. Živić et al. (2009) found that macroinvertebrates assemblages would mostly return to be similar to upstream assemblages at the distance of 3.5 km downstream from farm outfall. Thus, the distance of recovery appears to depend on a number of factors specific to the system. Doughty and McPhail (1995) determined that there was a recovery of macroinvertebrate assemblages within 19 months of the discharge ceasing which suggested the process of downstream recovery of benthic fauna might not happen if fish farms remained. The solution for that would be management and monitoring of waste discharge to diminish impacts on rivers.

4.4.1 Brumbys

There were clear differences in macroinvertebrate community composition between stations at the Brumbys. In all our sampling times, a clear separation in community composition was seen between the outlet and other stations. There were only slight differences between the three downstream stations over four seasons while communities of the two upstream

stations were similar to each other in summer and autumn; but were relatively different in winter and autumn. Community structure at each station was similar between summer and autumn as well as between winter and spring; supporting autumn and spring sampling schedule of the EPA to monitor the impacts of farm outfalls on river streams. Furthermore, upstream 1 and downstream 3 were close in the PCO plot as well as SIMPER analysis showed similarities in macroinvertebrate composition between those two stations; suggesting recovery process would occur at further downstream. However, the community of downstream 3, which was 800 m downstream from the outfall, did not return fully to upstream assemblages. Rather the downstream station displayed a combination of fauna found in both clean water and impacted sites. During sampling in spring, assemblages at each station were different to summer indicating the communities did not fully recover within one sampling year period.

Similarly, SIGNAL 2 index indicated the outlet station was the most polluted station over four seasons, which was classified as *severe pollution* (summer, autumn and winter) and *moderate pollution* (spring). This might be because of the compounding effect of fish farm effluent on top of upstream water which has already been impacted by agricultural practices. There were slight differences in water quality rating between the two upstream stations although upstream 1 appeared to be more polluted than upstream 2, which were generally moderate pollution and mild pollution respectively. The differences might be because upstream 1 is in agricultural land and upstream 2 is immediately above the outfall but the water runs through a weir and aquatic vegetation which would remove some of the nutrients which impacted upstream 1. Therefore, the selection of the control is quite important to these studies. The three downstream stations mostly rated moderate pollution over time, however, the level of

pollution decreased gradually from downstream 1 to 3. Moreover, no marked changes in SIGNAL 2 were seen at each station. Those suggested the communities within approximately 800 m downstream from the farm outfall did not return to upstream assemblages in one-year time.

In relation indicator species, Hydrobiidae, Hydropsychidae, Paramelitidae, Caenidae, Elmidae (L), Ceinidae, Elmidae (A) were indicative of upstream conditions with *mild pollution*. Oligochaeta, Chironominae, Glossiphoniidae, Hirudinea, Sphaeriidae, Glacidorbidae were indicators for the outlet station with severely impacted conditions. Moreover, SIMPER analysis detected that some pollution sensitive taxa (Baetidae, Elmidae, and Caenidae) were present at upstream; but were absent at the outlet and reduced at downstream stations; which is similar to the findings of previous studies on the effects of trout farms on streams (Brown and King, 1995; Camargo, 2019; Guilpart et al., 2012). These studies also asserted that the number of Oligochaeta, Simulidae and Chironomidae increased at the outlet and downstream; which were similar to the present results. Previous research also reported similar findings that the impacts of farm effluents resulted in a reduction of taxa richness as well as abundance of pollution intolerant taxa; but a rise of densities of pollution tolerant taxa (dos Santos Rosa et al., 2013; Doughty and McPhail, 1995; Loch et al., 1996a).

In relation to relative abundance, the lowest relative abundance was seen at the upstream 1 and outlet stations over four seasons with high number of dominant species whereas higher relative abundance was recorded at upstream 2 and other downstream stations. This might be because Brumbys is a big lowland river with slow flow and upstream 2 is just above the outlet; thus, this station could be enriched by organic waste from effluents.

Significant differences in ecological metrics (total abundance, family richness and Simpson diversity index) between the six stations was seen, but the patterns varied. Over four seasons, upstream 1 and downstream 3 had a lower total abundance and taxa richness, but higher diversity index compared to other stations. In contrast, higher total abundance and taxa richness with high number of dominant species made upstream 2, outlet and downstream 1 as stations which had lowest diversity index. The upstream 2 might have similar patterns to the outlet, but the species were different and it was the cleanest station within sampled stations which likely reflected river geomorphology. Specifically, upstream 1 is adjacent to agriculture while the water runs through a weir and aquatic vegetation at upstream 2 which would remove some agricultural nutrients. Furthermore, upstream 1 had lower taxa richness than that of the outlet and was less impacted with a higher diversity index. This finding was different to Selong (1997) who detected that a decrease or lower macroinvertebrate taxa richness was indicative of a degraded stream environment. However, community composition at the outlet contained more pollution tolerant species than at upstream 1, resulting in a better SIGNAL 2 index for upstream 1. This might be because the farm outfalls supplied waste nutrient which could supply as feed for invertebrates, which may slightly increase number of taxa but result in some dominant species. Downstream 2 had higher value in all those three categories with no particularly dominant species. The combination of these biological variables, relative abundance and signal 2 index suggested the level of pollution (from the most to least impacted) are the outlet station, downstream 2, downstream 1, downstream 3, upstream 1 and upstream 2. This again suggested that the relationship between biological pattern may relatively illustrate stream condition as PCO; illustrating the outfall is the worst station in the PCO analysis supported by lower taxa richness, diversity

index and SIGNAL 2 as well as more indicator species as pollution tolerant taxa compared to other stations.

4.4.2 Florentine

Macroinvertebrate communities of the outlet and downstream 1 stations appeared to be the most different from other stations over four sampling times. Similarities in community structure between two upstream station, between outlet and downstream 1, and between other three downstream stations were seen over time. Moreover, community composition at further downstream station (downstream 4) tended to be similar with upstream station (upstream 1), indicating that there was a gradient of recovery in the benthic community structure. Differences in communities at each station between two seasons of 2016 and 2017 also suggested invertebrate assemblages changed after the flood. This resulted in higher SIGNAL 2 scores which indicated a better water quality rating in 2017 (after flood) compared to 2016 samples. *Healthy habitat* was scored at the two upstream stations at all sampling times while outlet and all downstream stations were classified as *moderate pollution* in autumn 2016 and *mild pollution* in summer and autumn 2017. It could be suggested that the major flood swept away waste matter on the stream bed, resulting in better water quality ratings at downstream sites as Camargo (1994) and Camargo et al. (2005) determined that the changes and response of macroinvertebrates were related to organic pollution and nutrient enrichment. Moreover, downstream 1 seemed to be more polluted than the outlet as it was moderately polluted in two seasons of year 2016 while the outlet was classified as *mild pollution* in that year. Site scores increased slightly from downstream 2 to downstream 4 over the four times; suggesting water quality was improving moving downstream. Furthermore, PCO and SIGNAL 2 were useful in suggesting indicator species for different

levels of stream conditions from upstream to downstream. Psephenidae (water-penny beetles), Gripopterygidae (stoneflies), *Eusthenia costalis* (stoneflies), Ceinidae (amphipods), Elmidae (riffle bettles), Baetidae (mayflies), Paramelitidae (amphipods) and Leptoplebiidae (mayflies) were indicative of non-farming conditions (upstream stations). Oligochaeta (aquatic worms), Planorbidae (ramshorn snails), *Physa acuta* (air-breathing freshwater snails), Hirudinae (leeches) and Ancylidae (freshwater limpets) were indicators of polluted conditions at the outlet and downstream station just below the outlet. Orthocladiinae (midges), Tanypodinae (midges), Chironominae (midges) and Hydropsychidae (caddidflies) were indicative of *mild pollution* in stations with some level of impact downstream. This result is similar to the research of Selong and Helfrich (1998) and Camargo (1992a) which showed the abundance of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) declined downstream while the abundance of pollution tolerant such as Oligochaeta taxa increased.

There was a similar relative abundance between stations over the four sampling times. At each station, no significant differences in relative abundance were seen between four sampling times even though there was a slight decrease after the flood. A high number of dominant species were present at outlet, downstream 1 and downstream 3 stations. Moreover, pollution sensitive taxa (Baetidae, Leptophlebidae, *Costora Delora*, Conoesucidae, Leptoceridae) were more dominant at the downstream stations after the flood; suggesting better stream water quality in 2017 compared with 2016.

There were significant differences in ecological patterns (total abundance, family richness and Simpson diversity index) between stations over four sampling times. Regarding total abundance, no significant differences between downstream stations and upstream stations

were seen in summer 2016 while total abundance of downstream stations differed significantly from others in the other three times. In contrast, taxa richness between stations were similar over time. In relation to diversity index, there were differences between stations in year 2016, but not in year 2017. Moreover, a decreasing total abundance and taxa richness after the flood were seen at each station, except upstream 1 and downstream 2 whereas diversity index at each station rose after the flood; indicating there were fewer dominant species at each station after the flood. It suggested the food (solid waste) may have been a major impactor on biological changes at Florentine River. It may be explained by waste nutrients which supplied feed for invertebrates from farm outfall was flushed away by the flood. This would result in less food limiting the expansion of dominant species. Therefore, there was a reduction in total number of individuals as well as in number of dominant taxa. Taxa richness decreased very slightly at each station after the flood. As a result, higher diversity index was seen at each station after the flood as a light decrease in taxa with less dominant species resulting in higher diversity index compared to a slightly higher taxa richness but had dominant species. This suggested impacted stations (outlet and downstream 1 in summer and autumn 2016) had high total abundance and their relative abundance showed some dominant taxa; resulting in low diversity index compare to others.

In general, the major flood in 2016 (once in 100 year-time in Tasmania) had a positive influence on macroinvertebrates assemblages, stream water quality as well as biological conditions. This resulted in more pollution intolerant taxa in the community, better SIGNAL 2 indices (better quality rating), and higher diversity index at downstream stations. However, the community structure at downstream stations were not similar to upstream stations; suggesting the community did not fully recover to that of upstream conditions. Ideally the

sampling should have extended further downstream however access to this part of the river for sampling was not possible as the river became slower and deeper in nature.

4.5 Conclusion

In conclusion, we found that changes in macroinvertebrate abundance and community structure among stations at the two streams were associated with proximity to farm waste discharge but also likely reflected natural habitats surrounding the streams. The less disturbed river (Florentine) provided higher abundance of pollution intolerant taxa. A lower abundance of pollution intolerant taxa and different community structure occurred at the stations (outlet and downstream just below the outlet) closest to the waste discharge. Similarly, stream quality of the less disturbed stations (upstream and further downstream) was cleaner than the impacted stations (outlet and downstream next to the outlet) (as inferred by the Signal scores). Community structures of downstream stations were different from the outlet and upstream stations. The most impacted station was the farm discharge point, possessing a much higher number of pollution tolerant taxa and a lower number of taxa and a different community structure. Temporal comparison showed that macroinvertebrate assemblages at the Brumbys were more similar in summer and autumn and in winter and autumn. At the Florentine, the communities between summer and autumn 2016 were most similar but were also similar to that of autumn 2017. The community composition in summer 2017 (after the flood) was the most different from others sampling times at the Florentine, indicating the impacts of the flood on stream macroinvertebrates. Furthermore, a gradient of recovery in the macroinvertebrate community structure occurred further downstream from the outfall at each river although, the community structure did not fully recover within 800 m downstream of the outfall. This suggests further research on

recovery processes of downstream macroinvertebrates at stations further downstream to determine recovery points; which help farms monitor and diminish the farm outputs on receiving water.

5 Chapter 5: Is there a relationship between stream macroinvertebrate communities and physico-chemical water parameters?

Abstract

This study examined the relationship between macroinvertebrate communities and water quality parameters at seven sites in Tasmania during summer and autumn of 2016 and 2017 to assess whether macroinvertebrates can be used as a tool to monitor water quality. Principle Coordinates Analysis (PCO) and BEST (Bio-Env + Stepwise) were performed to identify the best correlation between macroinvertebrates and water quality variables. There were associations between macroinvertebrate community structure and water quality variable which separated the sites based on the level and type (grazing/agriculture and aquaculture farm) of impact. , The less disturbed sites had a higher abundance of pollution intolerant taxa (notably Psephenidae, Baetidae, Eusthenia costalis, Gripopterygidae, Atalophlebia australis, Costora Delora, Lingora sp. and Scirtidae) and dissolved oxygen and pH levels greater than 9 mg/l and 7 respectively which indicated good water quality. Those clean-water taxa decreased in abundance at the more polluted sites. Sites surrounded by agriculture and grazing had Tipulidae, Ceinidae, Paramelitidae, Caenidae, Hydrobiosidae, Ecnomidae, Sciomyzidae, Hydroptilidae, and Calamoceratidae associated with them and a higher conductivity and total nitrogen ranging from 0.2 – 0.6 mg/l and 280 – 570 ug/l respectively. In contrast, aquaculture sites tended to have Oligochaeta, Planorbidae, *Physa acuta*, *Cura sp.* and Hirudinae associated with them as well as markedly higher levels of nitrogen and phosphorus compared to other sites. Ammonia, nitrate, nitrite, nitrate & nitrite, total phosphorus concentrations at farm sites ranged from 210 – 580 ug/l, 0.06 – 0.17 mg/l, 0.011 – 0.25 mg/l, 72- 200 ug/l, and 40 – 80 mg/l respectively. Thus, the presence of certain

macroinvertebrates appears to indicate different types of impact and in general, macroinvertebrates appear to be quick, robust and cost-effective tools for aquaculture farms and government agencies wishing to assess and monitor aquatic systems. Nevertheless, further research needs to be undertaken to identify a single macroinvertebrate taxon or a small number of taxa which can provide the same information as groups of indicators.

5.1 Introduction

The use of macroinvertebrates as biomonitors is well known and a number of reviews describe how they can be applied to assess and manage environmental condition in rivers and streams (Azrina et al., 2006; Bennison et al., 1989; Berkman et al., 1986; Cairns Jr, 2017; Camargo, 1993; Chessman, 1995; Metcalfe, 1989; Spruzen et al., 2008; Whiles et al., 2000). In chapters 2 and 3 we have shown that in Tasmania, macroinvertebrates were very effective bioindicators of river "health" with the capacity to accurately identify impacts of a range of human activities including fish-farm activities, agriculture and grazing.

Water quality parameters have also been widely used to examine environmental impacts (Azrina et al., 2006; Camargo, 1993; Camargo, 1994; Fries and Bowles, 2002; Hardie et al., 2012; Pulatsu et al., 2004b). Changes in key indicators such as nutrients (nitrogen and phosphate) or dissolved oxygen can indicate significant deterioration in water quality (Elser et al., 2007; Jarvie et al., 1998; Kannel et al., 2007; Szmant and Forrester, 1996; Xu et al., 2010). Such changes in water quality will influence the types of plants and animals and as such, water quality and benthic ecology are inherently linked (Chow-Fraser et al., 1998). In Australia, measurement of water quality parameters and water chemistry is a requirement of the AUSRIVAS monitoring program (Parsons et al., 2002), and the EPA and as such is a key tool for the assessment and management of river health. For fish farms, water chemistry can

provide both an understanding of the nature of the farm outputs and the potential effects on receiving streams, and as such can provide the insights that will allow managers to develop a sustainable management plan for their particular system. Different macroinvertebrate taxa have different sensitivities to organic enrichment and pollutants, and consequently the benthic community will reflect the water quality and vice versa (Azrina et al., 2006; Camargo, 1994; Chessman, 1995; Goodnight, 1973; Metcalfe, 1989; Slooff, 1983). Many species will be negatively affected by changes in water quality, particularly elevated nutrients and any associated reduction in DO (Azrina et al., 2006; Camargo, 1994; Chessman, 1995; Goodnight, 1973; Metcalfe, 1989; Slooff, 1983), and so may not be able to survive in areas where such conditions occur leading to flow on changes in the local food-web and trophic interactions. Different taxa of macroinvertebrates consume different types of food such as organic and inorganic matter, algae, and aquatic plants. Therefore, changes in the macroinvertebrate community composition can reflect the impacts of fish farm feeds, organic matter, chemicals and various pollutants on the aquatic environment. Although many taxa respond negatively to higher nutrient released from fish farms, some taxa such as Oligochaeta, Chironomidae, Hirudinae can occur in higher abundance near fish farms (Goodnight, 1973; Kaeser and Sharpe, 2006; Paisley et al., 2011).

This study examined the relationship between macroinvertebrates and water chemistry (quality) in the Derwent River Catchment between 2016 and 2017 to assess how effective water chemistry might be as a proxy for the animals in Tasmania or if there might be instances where specific animals (invertebrate assessments) might actually be the best (most cost – effective) toll for a monitoring program e.g where identification of one animal can provide the same assessment as faunal or several chemistry assessment. The objectives of this study

were (1) to characterise the relationship between macroinvertebrates and specific water quality indices and (2) to confirm to what extent water chemistry might be able to replace macroinvertebrates (and vice versa) in a robust and cost-effective monitoring program.

5.2 Materials and Methods

5.2.1 Site selection

Macroinvertebrate sampling was undertaken at seven sites in the Derwent River Catchment where water quality sampling for the Derwent Catchment Program was conducted. Samples were collected from the Florentine River, Tyenna River at Russell Falls and Tyenna End (Tyenna River at the End), the Broad River, the Styx River, the Dee River and the Ouse River in summer 2016 (February 2016), autumn 2016 (April 2016), summer 2017 (January 2017) and autumn 2017 (March 2017) (see Fig 5.1). The Tyenna End site was located on the same river as the Russell Falls site but was much further downstream from the aquaculture inputs, and as such it was considered as a reference site for the Derwent Catchment Program.

Aquaculture farms were located on two rivers (the Florentine and the Tyenna at Russell Falls). There were two sampling sites in each of these streams; one upstream of the fish-farm inputs, which was considered an unimpacted site, and the other downstream of the farm outfall (approximately 200m downstream from the outlet), which was potentially an impacted site. Only one site was sampled in all of the other rivers, and this allowed us to assess the natural variability between streams.

Figure 5.1: The map of seven sampling sites in the Derwent Catchment (Google map, 2019)

Water quality parameters were measured by DPIPWE at all sites except Russel Falls, and at the same time as the invertebrate samples were collected. Tassal Pty Ltd collected and analysed the samples for the Russell Falls site. These included ammonia and ammonium as N ug/l, conductivity (Field) uS/cm (25 TRef), dissolved oxygen mg/L, dissolved oxygen percent saturation, filtered Phosphate as P ug/L, Kjeldahl Nitrogen total mg/L, Nitrate as N mg/l, Nitrite and Nitrate as N ug/L, Nitrite as N mg/l, Nitrogen (Total) as N ug/L, Non-Purgable Organic Carbon mg/L, pH field - sensor TC Units, Phosphorus (Total) as P ug/L, Salinity ppt, Total suspended solids (0.45um) mg/L, True Colour Hazen, Turbidity-Lab NTU, water temperature degrees C, and Chlorophyll-a.

At Russel Falls ammonia and ammonium as N mg/l, conductivity (Field) mS/cm (25 TRef), Filtered Phosphate as P mg/L, Kjeldahl Nitrogen total mg/L, Nitrate as N mg/l, Nitrite and Nitrate as N mg/L, Nitrite as N mg/l, Nitrogen (Total) as N mg/L, Non-Purgable Organic Carbon mg/L, pH field - sensor TC Units, Phosphorus (Total) as P mg/L, Total suspended solids (0.45um) mg/L, and Chlorophyll-a were measured.

The correlation between the macroinvertebrate and water quality data was done in two parts; firstly, by comparing the relationship between water quality and macroinvertebrates at unimpacted sites and secondly, by comparing the relationship between water quality and macroinvertebrates at the two aquaculture sites. Consequently, Part 1 included all sites except those upstream and downstream of Russell Falls and Part 2 only included the Florentine and Russell Falls sites.

5.2.2 Sampling and processing

This section is described as chapter 2

5.2.3 Data collection and analysis

5.2.3.1 Data collection

The total number of macroinvertebrate individuals, total number of each taxonomic group and the number of taxa were counted as described in chapter 2, while water quality data were provided by the DPIPWE and Tassal.

5.2.3.2 Data analyses

As only a single water quality sample was obtained by the DPIPWE for each site, the three replicate macroinvertebrate samples were averaged to provide a comparable single sample

for correlation analyses. Therefore, statistical analyses (PERMANANOVAs) were not performed as the requirement of minimum replicates was not met.

Comparison of the relationships between sites and times for macroinvertebrate and water quality data was undertaken using multivariate analysis (Principal coordinates analysis, PCO). For these analyses, macroinvertebrate data were square root transformed and Bray Curtis similarities calculated while water quality variables were normalised and Euclidean distance calculated. This provided two similarity matrices that could them be compared. Vector loadings of macroinvertebrates and water quality parameters were added onto graphical outputs to examine how well different sites and variables correlated. BEST (Bio-Env + Stepwise) analysis was performed to indicate the best correlation between the macroinvertebrate community structure and individual water quality variables (Clarke and Gorley, 2006b).

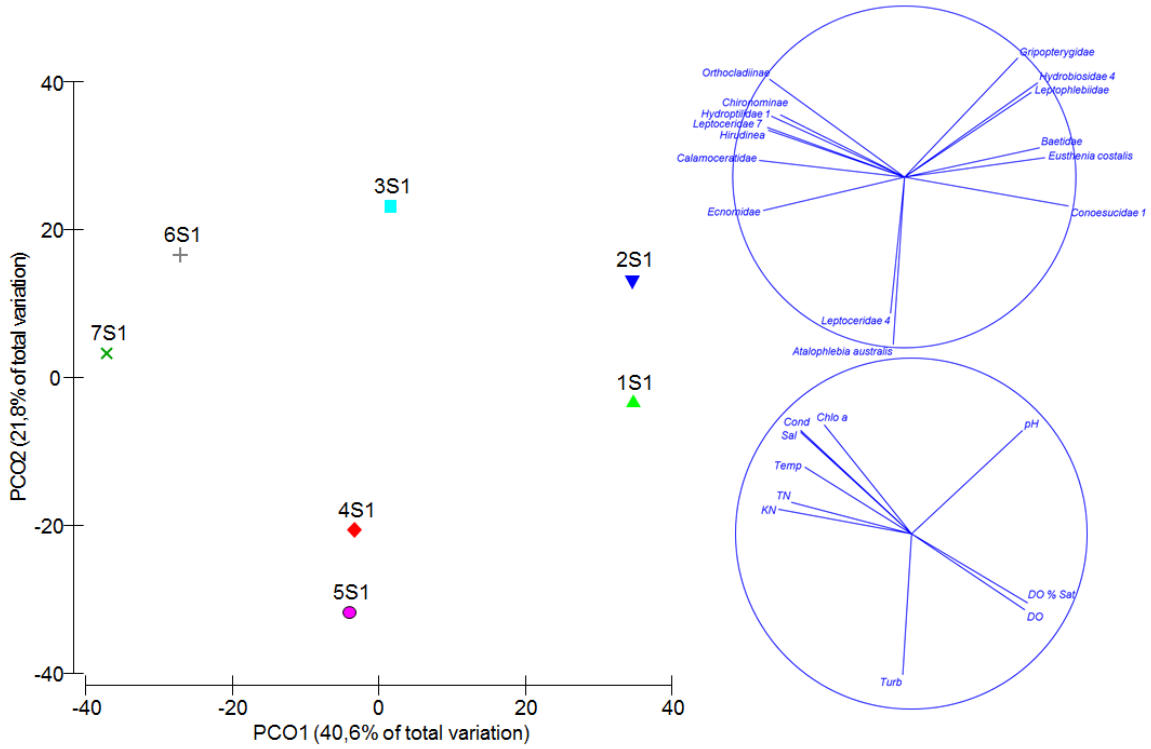
5.3 Results

5.3.1 Correlation between macroinvertebrates and water quality parameters at unimpacted sites

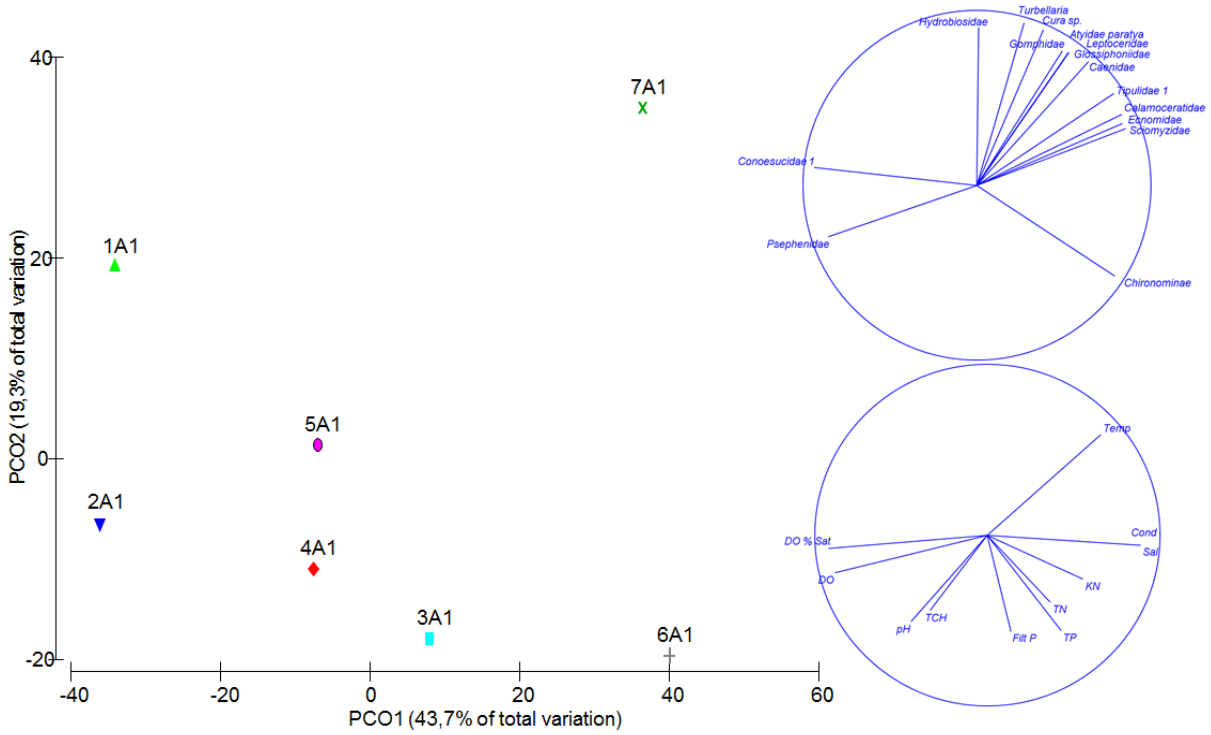
5.3.1.1 Correlation between macroinvertebrates and water quality parameters at seven sites each time

Principal coordinates analysis (PCO) clearly separates the 7 sites into 3 groups based on their macroinvertebrate community structure: group 1 included the Broad (1) and the Florentine 1 (2); group 2 included Florentine 2 (3), the Tyenna End (4) and the Styx (5); group 3 included the Dee (6) and the Ouse (7), Figure 5.2. Whilst there is some merging of sites within groups 1 and 2 over time, group 3 (the Dee (6) and the Ouse (7)) appear quite different to the other sites at all times. These two sites had a similar invertebrate community, but one which was very different to the other sites at all times. The macroinvertebrate community at the Florentine 2 (3), the Tyenna End (4) and the Styx (5) (Group 2) also remained quite similar over time but the samples collected in Summer and Autumn 2017 were quite similar to those from the Florentine 1 (2) Figure 5.2. Furthermore, the Florentine 2 (3) samples in summer 2016 were more different from the Tyenna End (4) and the Styx (5). Group 1, the Broad (1) and the Florentine 1 (2) could be readily distinguished from other sites in summer 2016 and autumn 2016; but as noted above, were similar to the Florentine 1 (2) in summer 2017 and autumn 2017.

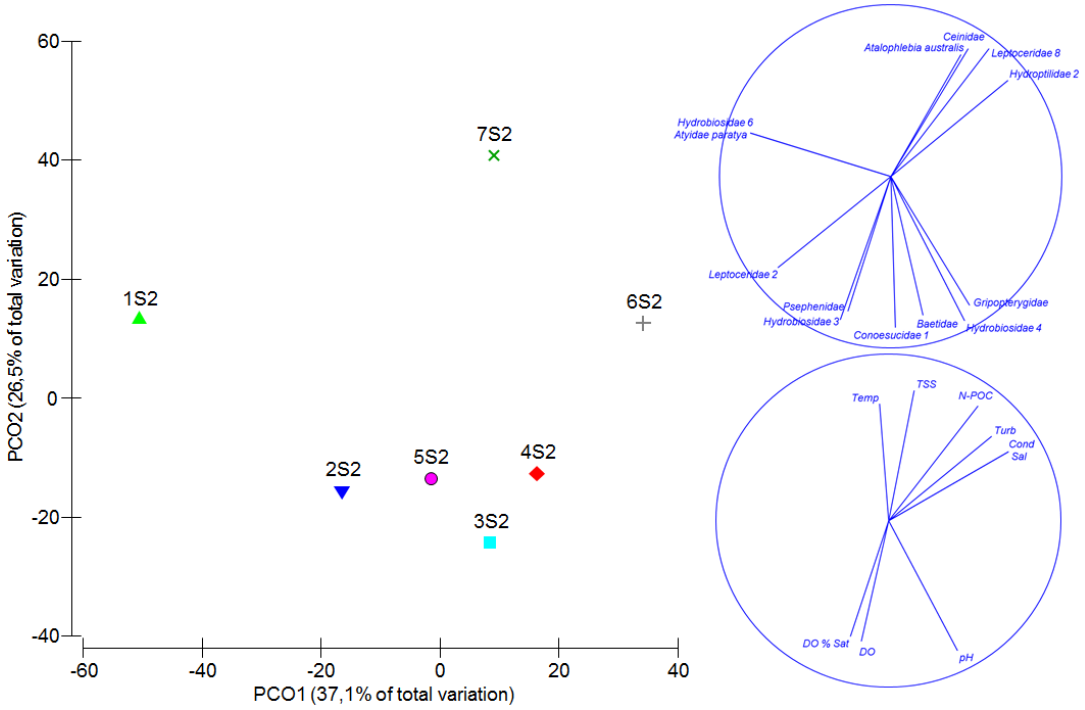
a) Summer 2016



b) Autumn 2016



c) Summer 2017



d) Autumn 2017

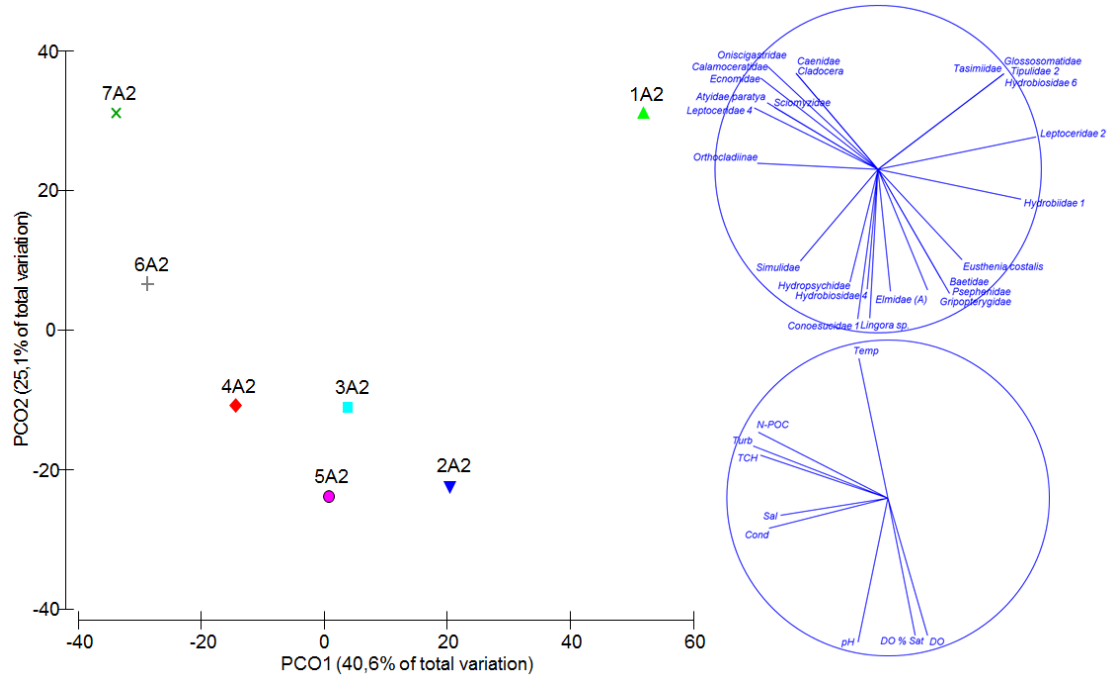


Figure 5.2: The two dimensional PCO plots showing relationships between sites in the Derwent River Catchment based on macroinvertebrate community structure.
Plots are arranged by time: a) summer 2016, b) autumn 2016, c) summer 2016 and d) autumn 2017. Vector overlays show both the dominant macroinvertebrate taxa for each plot and the key water quality (WQ) parameters driving the community separations (note the WQ parameters are restricted to those having vectors longer than 0.5). Site codes: 1: Broad, 2: Florentine 1, 3: Florentine 2, 4: Tyenna End, 5: Styx, 6: Dee, 7: Ouse.

Generally, the vector loadings suggested some sites consistently had high loadings for certain taxa across times (e.g. Calamoceritidae and Ecnomidae loaded strongly on the Dee (6) and the Ouse (7) in 3 out of 4 seasons) but most other sites had different taxa with relatively high loadings at different times. Similarly, the Broad (1) and especially the Florentine 1 (2) had high loadings for DO, %DO and the Dee (6) and the Ouse (7) had high loadings for conductivity, salinity and temperature. The other sites were more variable with different water quality parameters having high loadings at different times.

In summer 2016, vector loading for Conoesucidae 1 (caddisfly larvae) was positively correlated with the Broad (1) while Gripopterygidae (stonefly larvae), Hydrobiosidae 4 (caddisfly larvae), Leptophlebiidae (mayfly larvae) were strongly associated with the Florentine 1 (2). Moreover, Baetidae (mayfly larvae) and *Eusthenia costalis* (stonefly larvae) were correlated with those two sites. In proportion to invertebrates, higher level of pH, dissolved oxygen (DO) and DO percent saturation (DO % Sat) were associated with the Broad (1) and the Florentine upstream (2). *Atalophlebia australis* (mayfly larvae) and Leptoceridae 4 (caddisfly larvae) were highly associated with the Tyenna End (4) and the Styx (5) while turbidity was higher at those two sites. Calamoceratidae (caddisfly larvae) highly correlated with the Ouse (7) whereas Orthocladiinae (midges), Chironominae (midges), Hydroptilidae 1 (caddisflies), Leptoceridae 7 and Hirudinea (leeches) were associated with the Dee (6); which was higher in total nitrogen (TN) and Kjeldahl Nitrogen total (KN) as well as in Chlorophill-a, conductivity, salinity and temperature respectively. On the other hand, BioEnv analyses illustrated that the four water quality variables which best explained the pattern in the invertebrate community were the high levels of pH, temperature, conductivity and KN.

In autumn 2016, Psephenidae (water-penny beetle larvae) and Conoesucidae 1 as well as higher DO and DO % Sat were correlated with the Broad (1) and the Florentine 1 (2). Furthermore, pH and true colour hazen (TCH) Sat closely correlated with Florentine 1 (2). While Calamoceratidae, Ecnomidae (caddisfly larvae), Sciomyzidae (marsh fly larvae), Tipulidae (large crane fly larvae), *Atyidae paratya* (freshwater shrimp), Caenidae (small squaregill mayfly larvae), Hydrobiosidae (caddisfly larvae), Turbellaria (flatworms) and *Cura sp.* (flatworms) positively correlated with the Ouse (7); only Chironominae was associated with the Dee (6). Temperature was associated with the Ouse (7) whereas a higher level of Filt Phosphate (Filt P), total phosphorus (TP), KN and total nitrogen (TN) were associated with the Florentine 2 (3), the Tyenna (4) and the Styx (5). Furthermore, the BEST showed that higher DO, DO% sat, KN, salinity and temperature best correlated with separation of the community between sites.

In summer 2017, high levels of pH, salinity and N-POC were the three variables which best explained the separation of community (the BEST analysis). Particularly, vector loadings for Hydrobiosidae 6 and *Atyidae paratya* were correlated with the Broad (1) whereas Leptoceridae 2, Psephenidae, Hydrobiosidae 3 as well as DO and DO % sat of water quality were closely associated with the Florentine 1 (2). At Florentine 2 (3), Gripopterygidae, Hydrobiosidae 4, Baetidae and Conoesucidae 1 were associated with higher level of pH. Higher temperature, Total suspended solids (TSS), Non-Purgable Organic Carbon (N-POC), turbidity, conductivity and salinity were associated with *Atalophlebia australis,* Ceinidae (amphipods), Leptoceridae 8 and Hydroptilidae 2 (caddisfly larvae) at the Ouse (7).

In autumn 2017, Tasimidae, Glossomatidae, Tipulidae 2 and Hydrobiosidae 6 were associated with the Broad (1), *Eusthenia costalis,* Baetidae, Psephenidae, Gripopterygidae were

associated with the Florentine 1 (2) while only Simulidae (black fly larvae) were associated with higher salinity and conductivity at the Tyenna End (4). Moreover, those two sites were also associated with high levels of DO and DO % Sat. Hydropsychidae (filtering caddisfly larvae), Hydrobiosidae 4, Conoesucidae 1, *Lingora sp.* (caddisfly larvae) and Elmidae adults (riffle beetles). Caenidae, Cladocera (water fleas), Ceinidae, Calamoceratidae, Ecnomidae (caddisfly larvae), Atyidae paratya, Leptoceridae 4, Orthocladiinae (midges), Sciomyzidae were associated with the Styx (5) as were high levels of N-POC, turbidity, TCH and temperature at the group of the Dee (6) and Ouse (7). Within those water quality variables, high DO% sat, pH, TCH and conductivity described the best correlation between biological and individual variables (BEST analysis).

5.3.1.2 Correlation between macroinvertebrates and water quality parameters at each site across four times

PCO illustrates the separations of macroinvertebrate communities between the four sampling times at each site (Figure 5.3) with differences in the abundance of each taxa within the communities resulting in seasonal changes in macroinvertebrate structure at each site. Nonetheless, certain pollution tolerant taxa were consistently associated with high nutrient loads at impacted sites suggesting they were useful as indicators. Moreover, there were associations between water quality factors and macroinvertebrates in some seasons at all sites; even there were no clear associations between invertebrates or water quality with each season at each site.

At the Broad (1); Hydrobiosidae 6 were associated with temperature in summer 2017. In autumn 2017, nitrate, nitrite & nitrate, salinity, filt P and Chlorophill-a were associated with

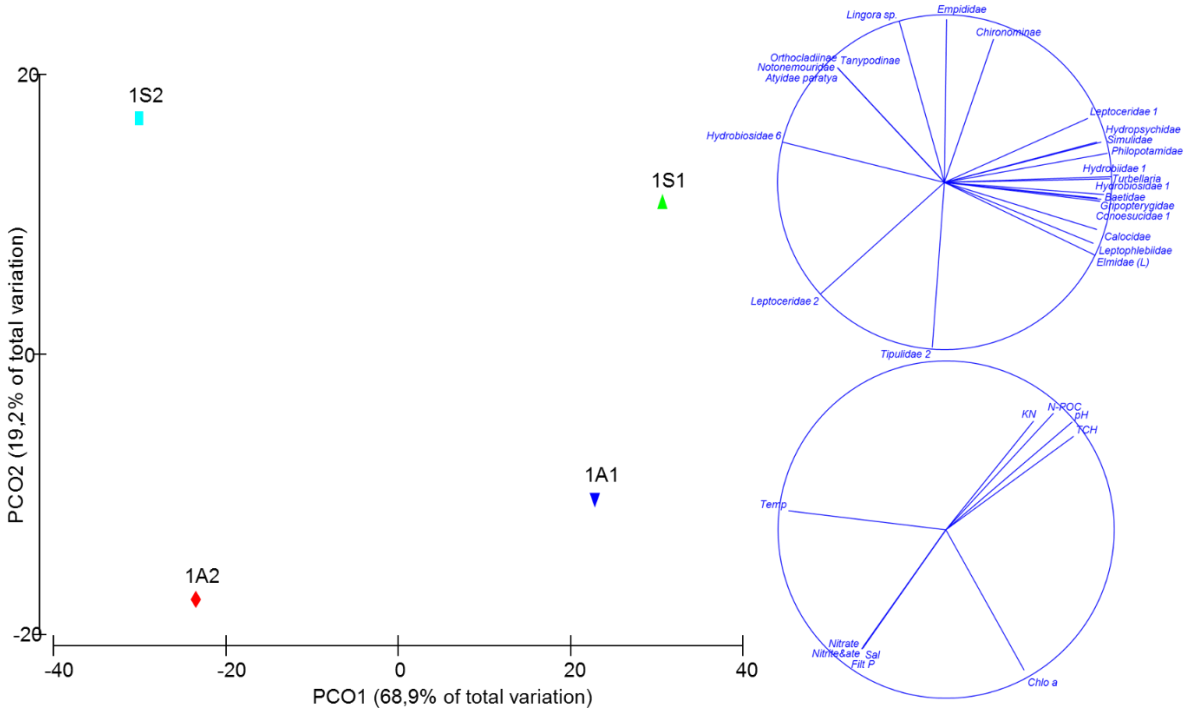
Leptoceridae 6 and Tipulidae 2 (crane fly larvae). However, the BEST showed that only temperature and TCH were the two variables which best explained the community pattern.

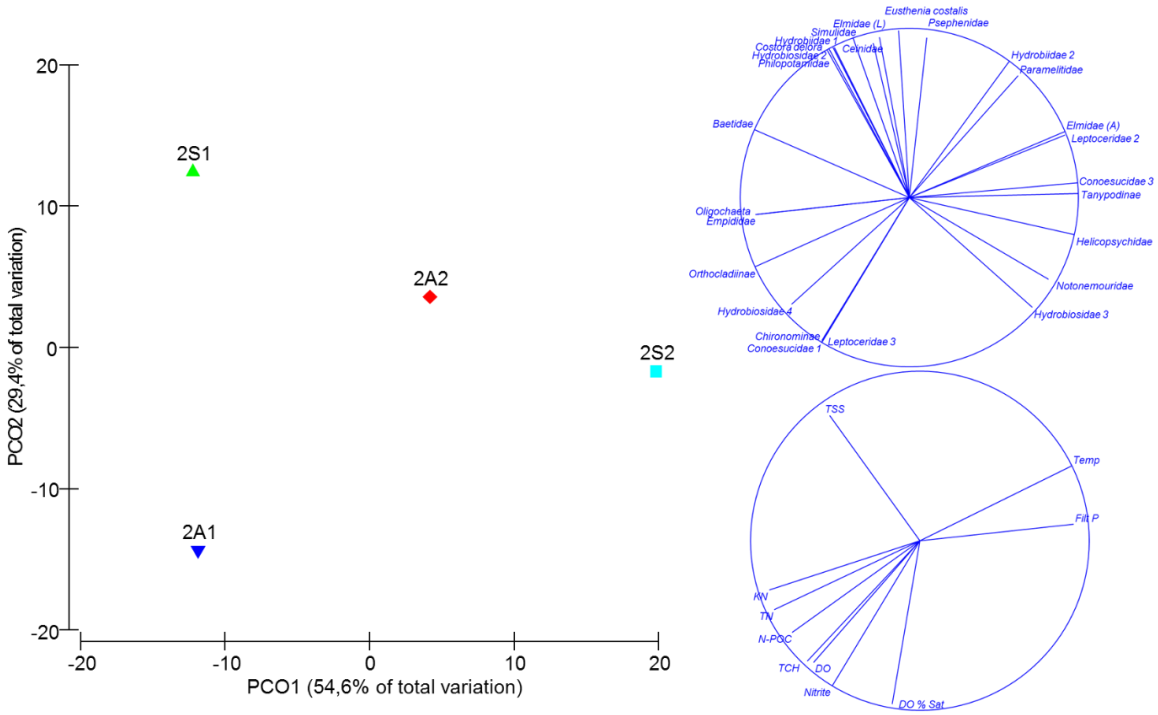
Upstream of the Florentine (2); in summer 2016, Philopotamidae (caddisfly larvae), Hydrobiidae 1 (mud snails), Hydrobiosidae 2, *Costora Delora* (caddisfly larvae), Ceinidae and Simulidae were associated with high TSS. In autumn 2016, Hydrobiosidae 1, Chironominae (midges), Conoesucidae 1, Leptoceridae 3 were associated with higher nitrite, TN, KN, DO, TCH and N-POC. In summer 2017, Conoesucidae 3, Tanypodinae (midges) and Helicopsychidae (snail-case caddisfly) were associated with high level of filt P whereas in autumn 2017, Paramelitidae (amphipods), Hydrobiidae 2 were associated with high temperatures. The three variables which the best explained with the community pattern were DO, pH and temperature.

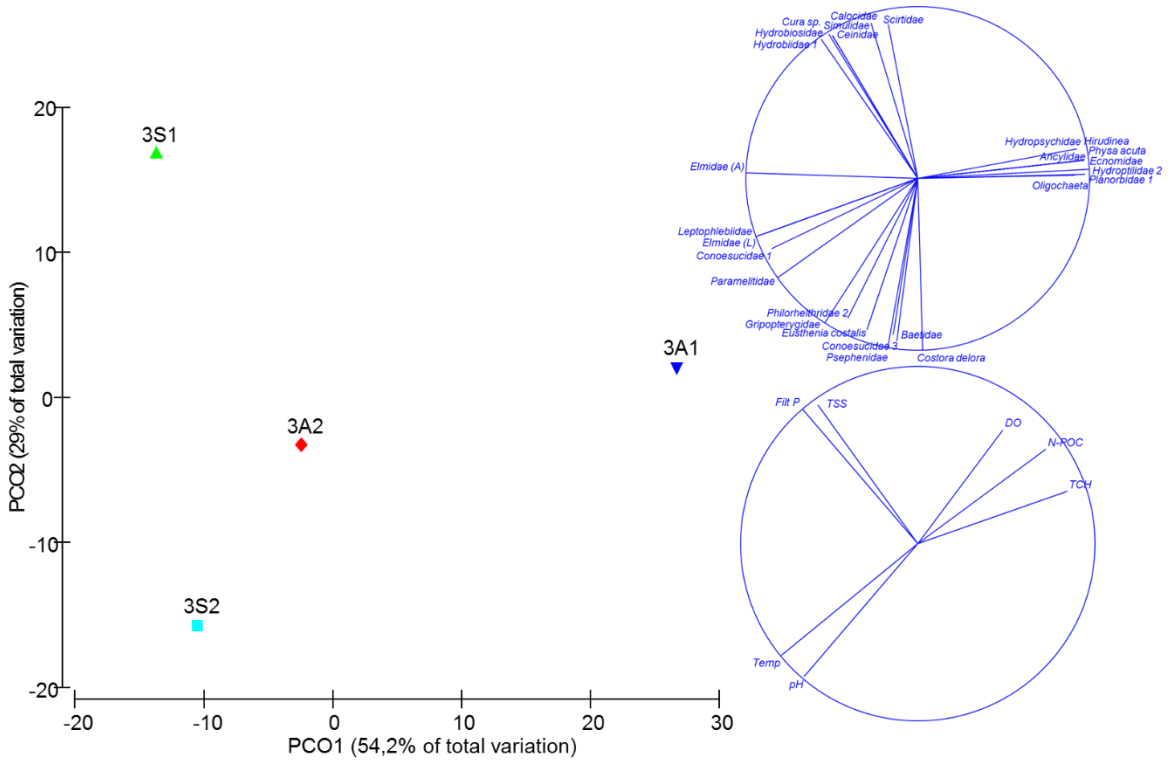
Downstream of Florentine (3), *Cura sp.* (flatworms), Simulidae, Hydrobiidae 1, Hydrobiosidae and Caenidae were associated with higher TSS, filt P in summer 2016. In autumn 2016, Hirudinae (leeches), Physa acuta (snails), Ecnomidae, Hydroptilidae 2, Planorbidae 1 (ramshorn snails) and Oligochaeta (aquatic worms) were correlated with high levels of N-POC, TCH, DO. Moreover, summer and autumn 2017 had higher pH and temperature; associated with Gripopterygidae, Philorheithridae 2, Paramelitidae, *Eusthenia costalis*, Conoesucidae 3, Psephenidae and Baetidae. Moreover, high levels of filt P, Nitrate, N-POC, TSS and TCH were the best correlated with the community which separated sites between times at the Florentine 2 (3).

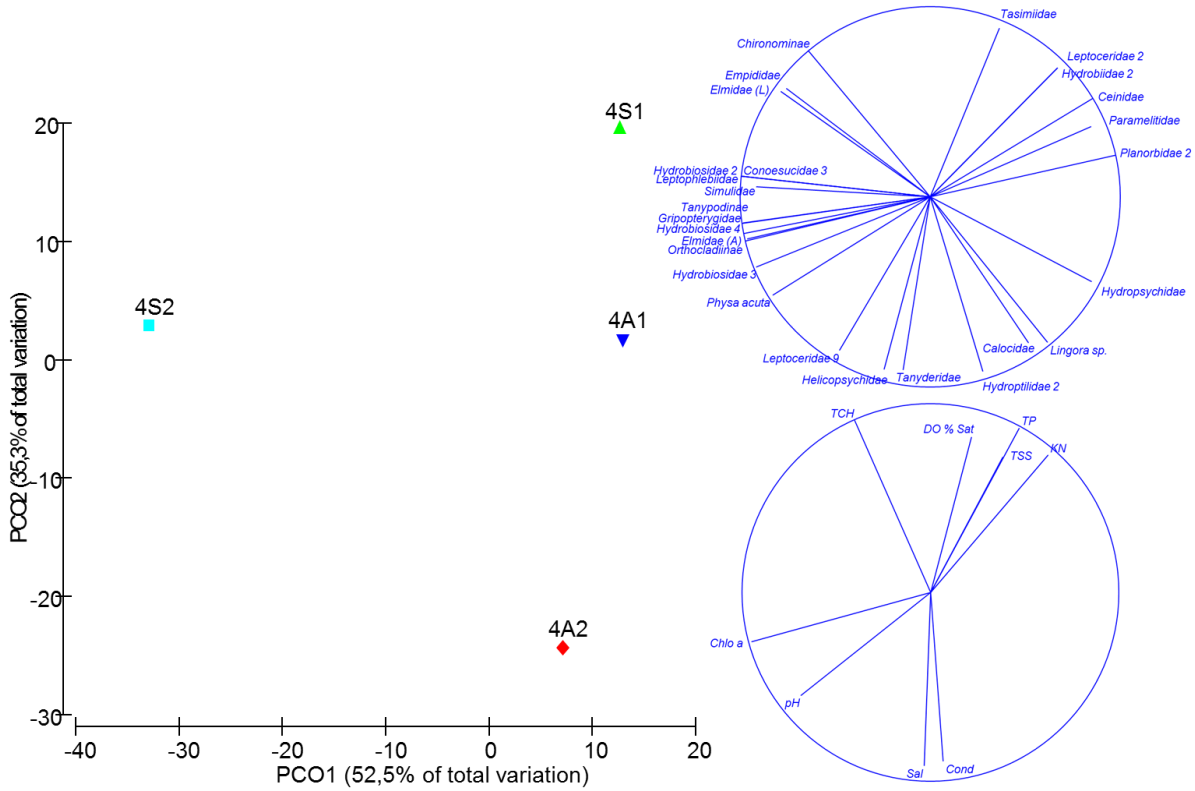
At the Tyenna End (4), Tasimiidae (caddisfly larvae), Leptoceridae 2, Hydrobiidae 2, Planorbidae 2, Ceinidae and Paramelitidae associated with high TSS, DO % sat, TP, KN in

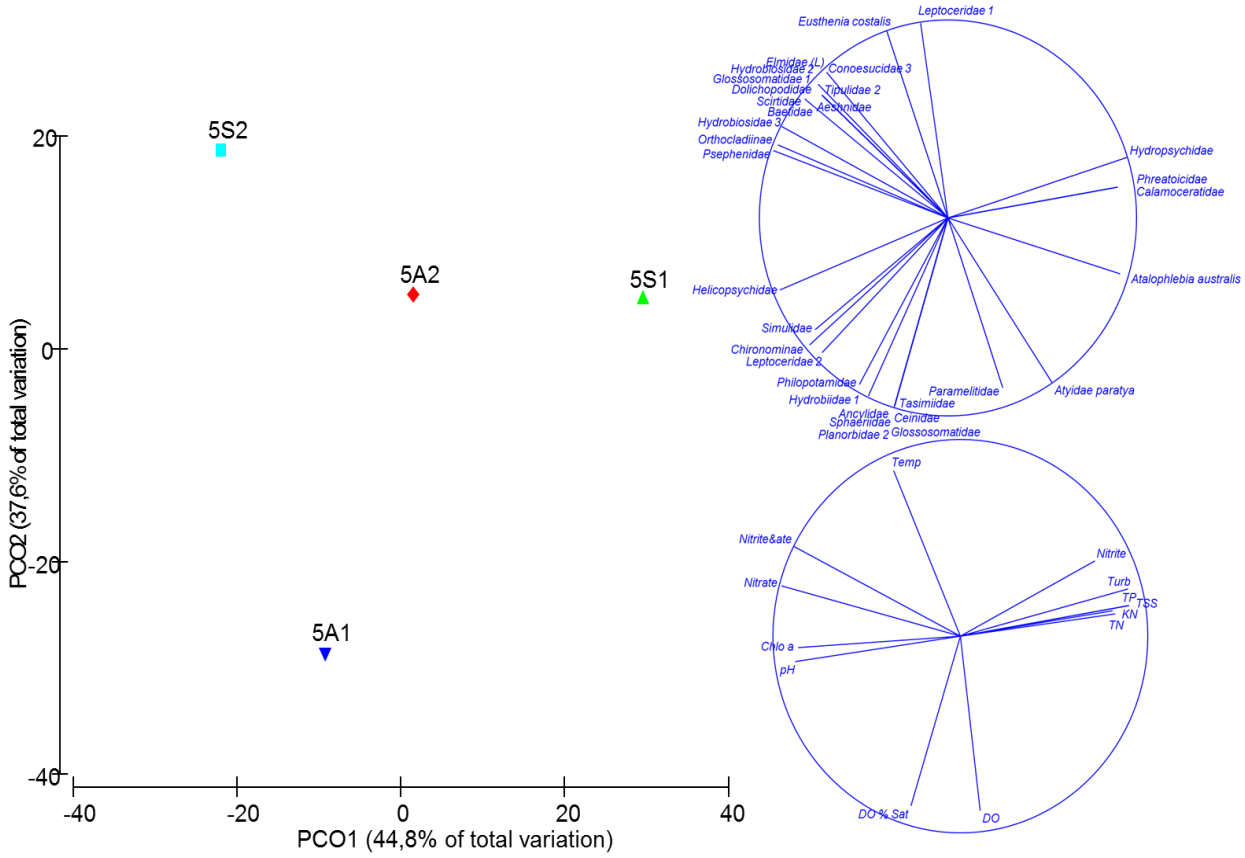
summer and autumn in 2017. Chlorophyll-a explained the best the separation at the Tyenna End (4) (BEST analysis).

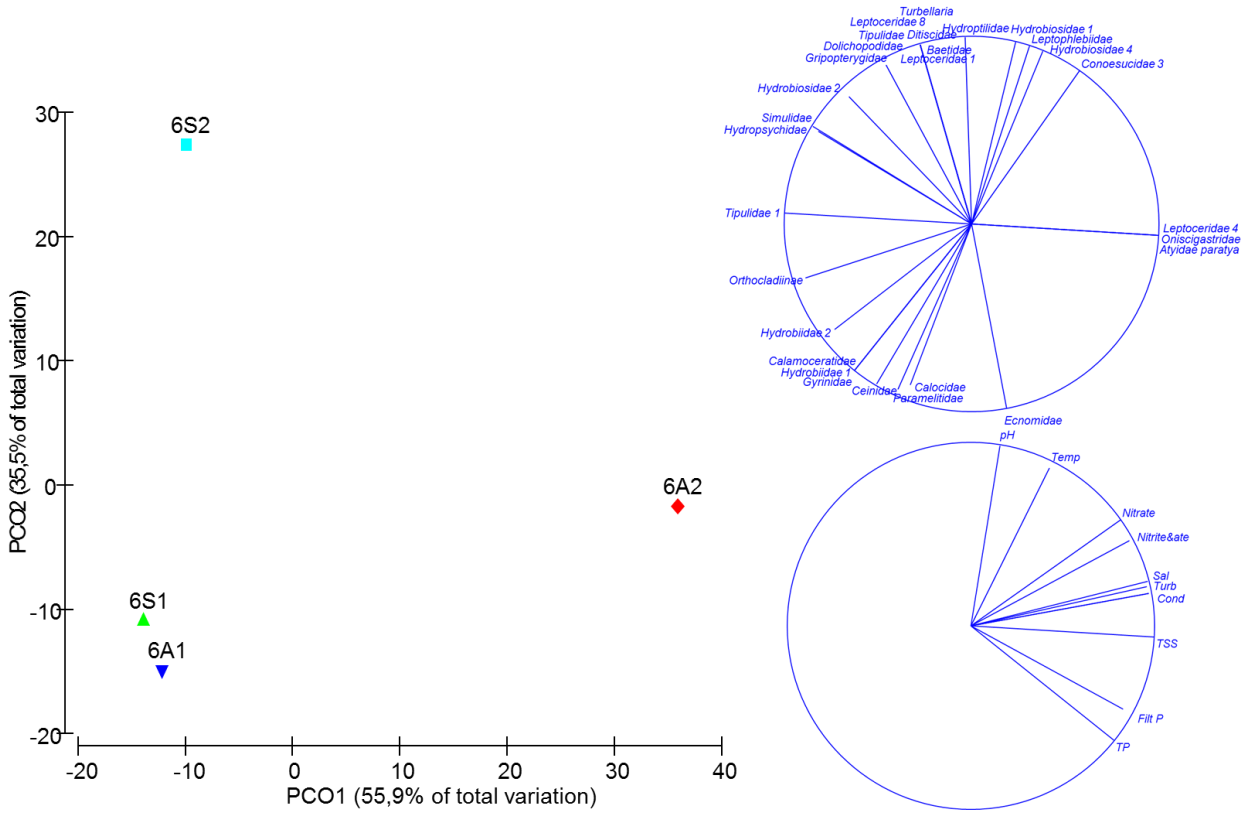





3S1
3A1
3A2
3S2
PCO2 (29% of total variation)
PCO1 (54,2% of total variation)
20
10
0
-10
-20
-20
-10
0
10
20
30
Cura sp.
Calcidae
Simulidae
Scirtidae
Hydrobiosidae
Ceinidae
Hydrobiidae 1
Hydropsychidae
Hirudinea
Physa acuta
Ancylidae
Ecnomidae
Hydroptilidae 2
Planorbidae 1
Elmidae (A)
Oligochaeta
Leptophlebiidae
Elmidae (L)
Conoesucidae 1
Paramelitidae
Philorheithridae 2
Gripopterygidae
Eusthenia costalis
Baetidae
Conoesucidae 3
Psephenidae
Costora delora
Filt P
TSS
DO
N-ROC
TCH
Temp
pH


4S1
4S2
4A1
4A2
PCO2 (35,3% of total variation)
PCO1 (52,5% of total variation)
20
10
0
-10
-20
-30
-40
-30
-20
-10
0
10
20
Tasimiidae
Chironominae
Leptoceridae 2
Empididae
Hydrobiidae 2
Elmidae (L)
Ceinidae
Paramelitidae
Planorbidae 2
Hydrobiosidae 2
Conoesucidae 3
Leptophlebiidae
Simulidae
Tanypodinae
Gripopterygidae
Hydrobiosidae 4
Elmidae (A)
Orthocladiinae
Hydrobiosidae 3
Hydropsychidae
Physa acuta
Calocidae
Lingora sp.
Leptoceridae 9
Helicopsychidae
Tanyderidae
Hydroptilidae 2
TCH
DO % Sat
TP
TSS
KN
Chlo a
pH
Sal
Cond


5S2
5A2
5S1
5A1
PCO2 (37,6% of total variation)
PCO1 (44,8% of total variation)
Eusthenia costalis
Leptoceridae 1
Elmidae (L)
Hydrobiosidae 2
Conoesucidae 3
Glossosomatidae 1
Dolichopodidae
Tipulidae 2
Scirtidae
Aeshnidae
Baetidae
Hydrobiosidae 3
Orthocladiinae
Psephenidae
Hydropsychidae
Phreatoicidae
Calamoceratidae
Atalophlebia australis
Helicopsychidae
Simulidae
Chironominae
Leptoceridae 2
Philopotamidae
Hydrobiidae 1
Ancylidae
Sphaeriidae
Planorbidae 2
Ceinidae
Tasimiidae
Paramelitidae
Atyidae paratya
Glossosomatidae
Temp
Nitrite&ate
Nitrite
Nitrate
Turb
TP
TSS
KN
TN
Chlo a
pH
DO % Sat
DO


6S2
6A2
6S1
6A1
PCO2 (35,5% of total variation)
PCO1 (55,9% of total variation)
Turbellaria
Leptoceridae 8
Hydroptilidae
Hydrobiosidae 1
Tipulidae Ditiscidae
Leptophlebiidae
Dolichopodidae
Hydrobiosidae 4
Gripopterygidae
Baetidae
Leptoceridae 1
Conoesucidae 3
Hydrobiosidae 2
Simulidae
Hydropsychidae
Tipulidae 1
Leptoceridae 4
Oniscigastridae
Atyidae paratya
Orthocladiinae
Hydrobiidae 2
Calamoceratidae
Hydrobiidae 1
Gyrinidae
Ceinidae
Calocidae
Paramelitidae
Ecnomidae
pH
Temp
Nitrate
Nitrite&ate
Sal
Turb
Cond
TSS
Filt P
TP

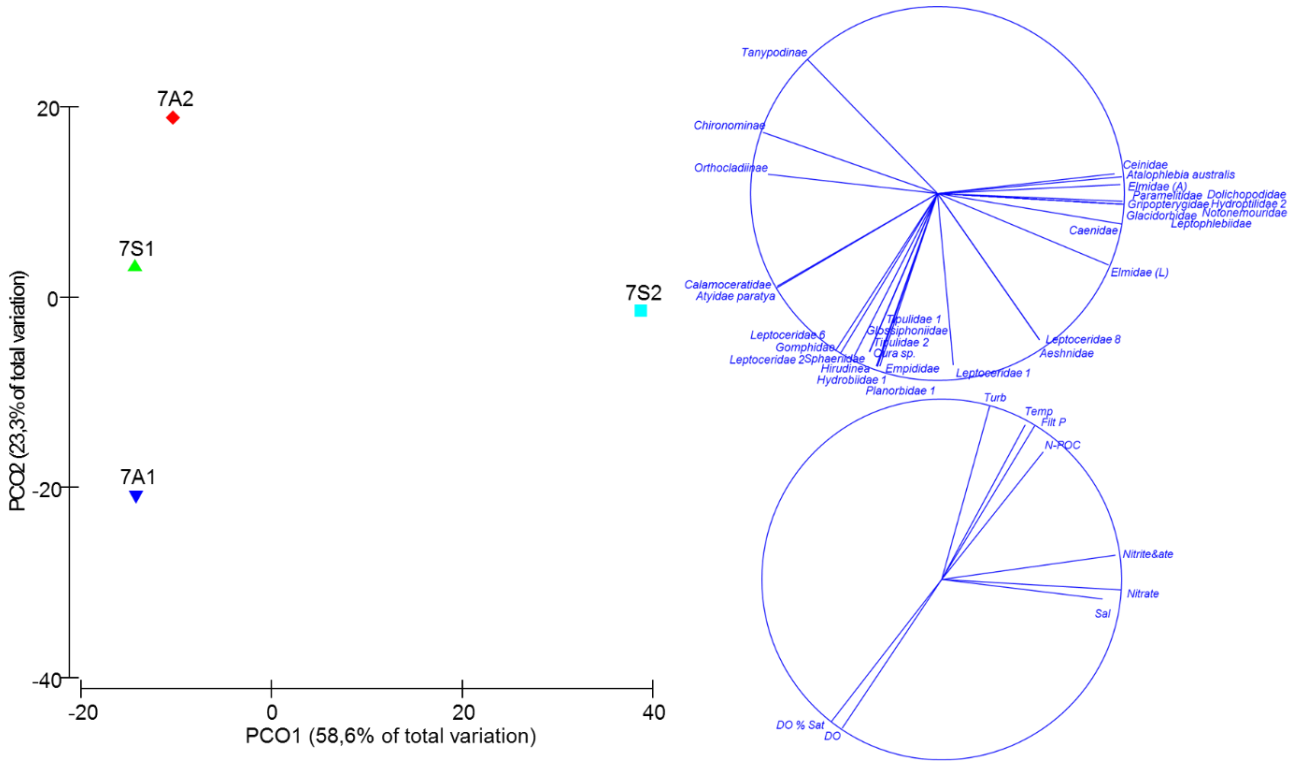


Figure 5.3: The two dimensional PCO plot showing relationships between four sampling times at each site based on macroinvertebrate community structure (S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017). Vector overlays show both the dominant macroinvertebrate taxa and the key water quality parameters
(note the WQ parameters are restricted to those having vectors longer than 0.5). Site codes: 1: Broad, 2: Florentine 1, 3: Florentine 2, 4: Tyenna End, 5: Styx, 6: Dee, 7: Ouse

At the Styx (5); Hydropsychidae, Phreatoicidae (freshwater isopods) and Calamoceratidae were associated with high levels of nitrite, KN, TN, TP, TSS and turbidity in summer 2016 whereas Paramelitidae, *Atyidae paratya*, Tasimiidae, Ceinidae and Glossosomatidae were associated with higher DO and DO % sat in autumn 2016. The BEST result showed DO%, turbidity and nitrite&nitrate the best explained the separation of the community.

At the Dee (6), conductivity, filt P and pH (the BEST analyses) were the three variables which explained the separation between seasons. The presence of high numbers of Hydrobiosidae 1&4, Leptophlebiidae and Conoesucidae 3 were associated with higher pH and higher water temperature in summer 2017 whilst Leptoceridae 4, Caenidae and *Atyidae paratya* were correlated with higher TSS, higher filt P and TP in autumn 2017.

At the Ouse (7), Leptoceridae 2&6, Gomphidae, Sphaeriidae, Hirudinea were associated with higher DO and DO % sat in autumn 2016 whereas nitrite & nitrate, nitrate and salinity were high in summer 2017 and associated with Caenidae, Ceinidae, Paramelitidae, Glacidoebidae, Hydroptilidae 2, Notonemouridae. Nevertheless, only DO% Sat and nitrate were the best correlated with the pattern in the community.

5.3.2 Correlation between macroinvertebrates and water quality parameters between stations upstream and downstream of two aquaculture sites

5.3.2.1 Correlation between macroinvertebrates and water quality parameters between upstream and downstream stations in two aquaculture sites in each season

Principal coordinates analysis clearly separates two downstream sites from two upstream sites; although communities of four sites could be differentiated over the four times (Figure 5.4). Moreover, the PCOs illustrate that macroinvertebrate community at downstream sites on each river was usually more similar to the other downstream site compared to the upstream site on the same river. Vector loadings for macroinvertebrates and water quality showed the correlation of invertebrates and water quality were not consistent with both specific season and site.

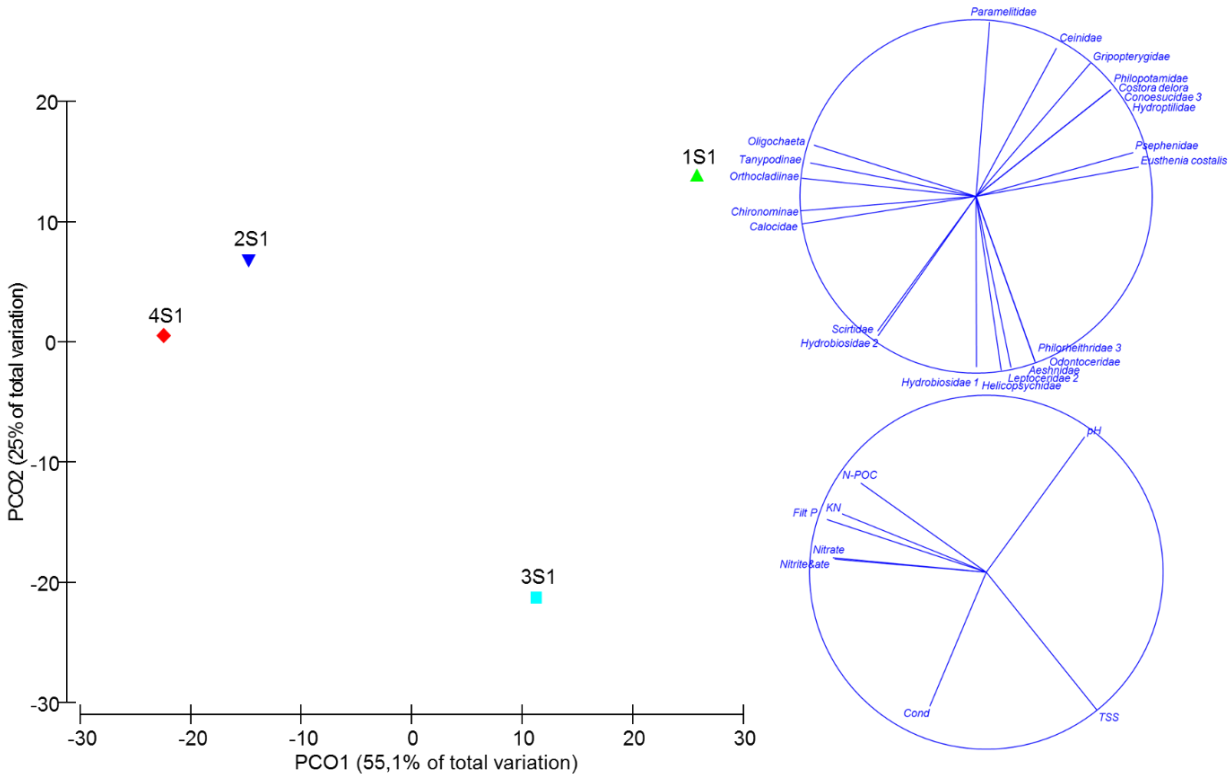
In summer 2016, the correlation between high level of pH and Gripopterygidae, Philopotamidae, Conoesuciade 3, Hydroptilidae, *Costora Delora* and Ceinidae was seen at the upstream site of the Florentine (1) while high TSS was correlated with Philorheithridae 3 (caddisfly larvae), Odontoceridae (caddisfly larvae), Aeshnidae (dragonfly larvae) and Leptoceridae 2 at the upstream site of the Russell Falls (3). In contrast, Oligochaeta, Tanypodinae, Orthocladiinae and Chironominae were abundant at downstream sites (2 and 4), especially at the downstream site of Russell Falls (4) and associated with higher levels of

N-POC, filt P, KN, nitrite&nitrate, and nitrate at those sites. BioEnv analyses suggested nitrite best explained the separation of the community.

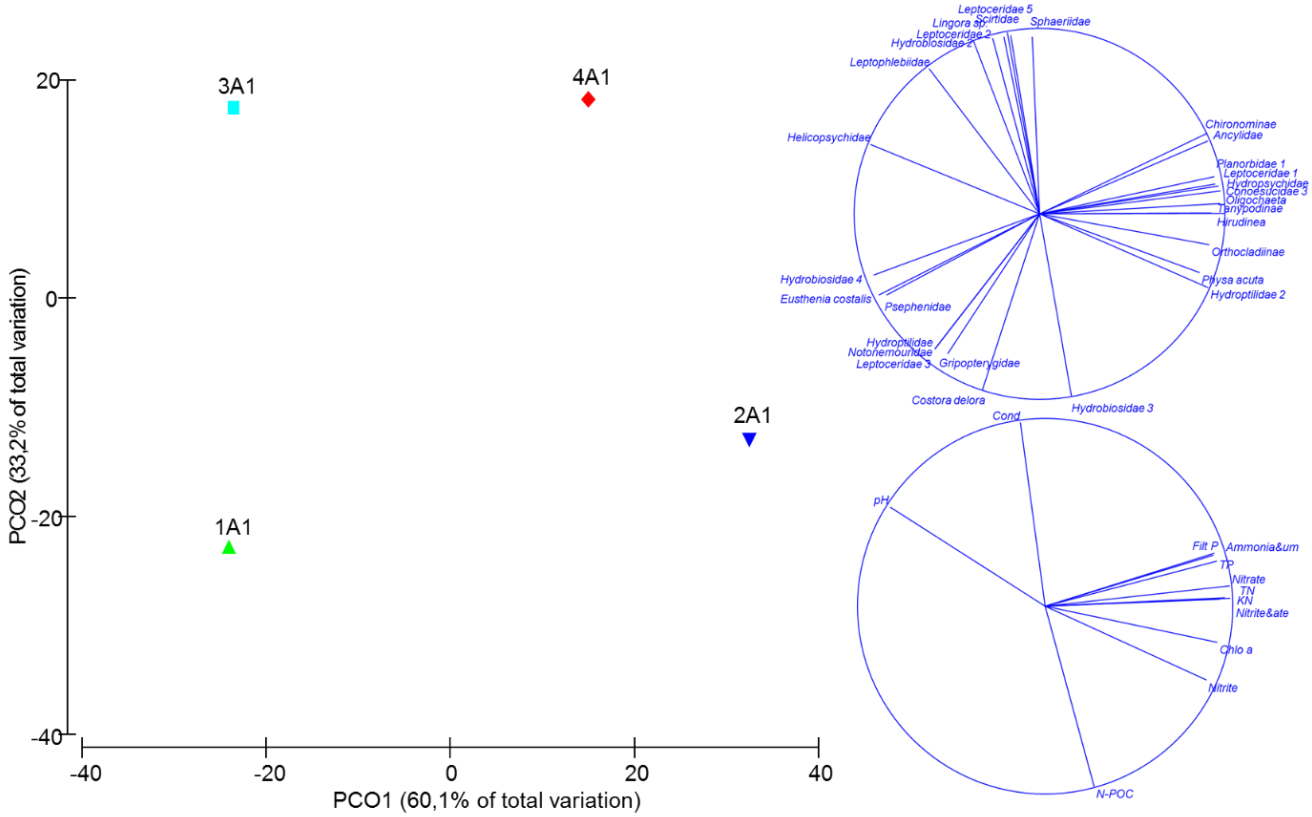
In autumn 2016 at the Russell Falls upstream site (3), higher conductivity and higher pH were correlated with the presence of Hydrobiosidae 2, Leptoceridae 2, *Lingora sp*. (caddisfly larvae), Scirtidae (water beetle larvae) and Leptoceridae 5. At the downstream site of the Russell Falls farm outfall (4); Oligochaeta, Chironominae, Ancylidae (air-breathing limpets) Planorbidae 1, Leptoceridae 1, Hydropsychidae, Tanypodinae, Hirudinae, Orthocladiinae, *Physa acuta*, and Hydroptilidae 2 positively associated with high levels of phosphorus, TP, ammonia and ammonium, nitrate, nitrite&nitrate, KN, TN and chlorophyll-a. The two variables which best correlated with the community patterns between sites were nitrite and filt P.

In summer 2017, *Eusthenia costalis*, Psephenidae, *Costora delora*, Conoesucidae 1, Hydrobiidae 2, Paramelitidae and Conoesucoidae 3 were associated with the two upstream sites (1 and 3) which was high in pH (alkaline). For the two downstream sites (2 and 4), the presence of Leptophlebiidae, Oligochaeta, Baetidae, Leptoceridae 1&7, Hydrobiosidae 4, Planorbidae 1 were correlated with high levels of nitrite, nitrate, nitrite & nitrate, KN, TN, ammonia & ammonium, filt P, TP, N-POC and Chlorophyll-a.

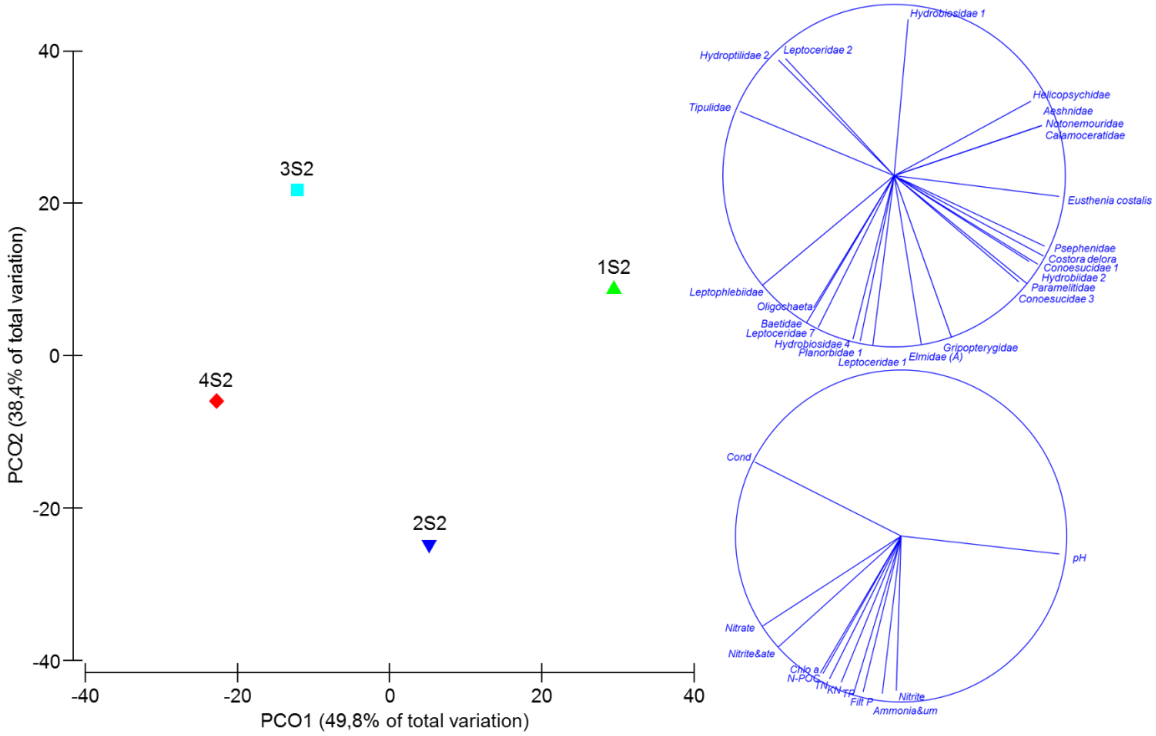
a) Summer 2016



b) Autumn 2016



c) Summer 2017



d) Autumn 2017

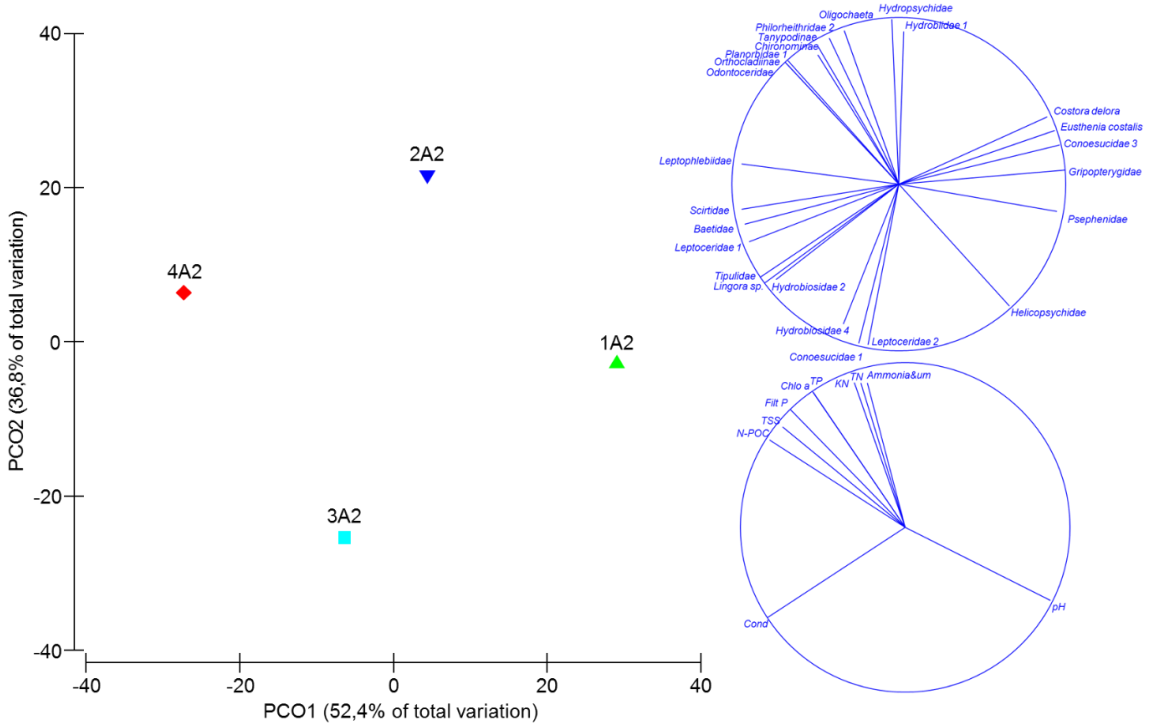


Figure 5.4: The two dimensional PCO plot showing relationships between sites based on macroinvertebrate community structure. Plots are arranged by time: a) summer 2016, b) autumn 2016, c) summer 2016 and d) autumn 2017. Vector overlays show both the dominant macroinvertebrate taxa for each plot and the key water quality (WQ) parameters driving the community separations (note the WQ parameters are restricted to those having vectors longer than 0.5). (1: upstream of farm at the Florentine, 2: right at farm outlet at the Florentine, 3: upstream of farm in Russell Falls, 4: closest to farm outlet at Russell Falls)

Similarly, the associations between water quality and macroinvertebrates were seen at the two downstream sites (2 and 4) in autumn 2017. The presence of pollution tolerant taxa including Oligochaeta, Tanypodinae, Chironominae, Planorbidae 1, Orthocladiinae, Odontoceridae and Philorheithridae 1 were associated with downstream sites (2 and 4); in which KN, TN, ammonia&ammonium, filt P, TP, TSS, N-POC and Chlorophill-a had substantially higher value. Furthermore, conductivity and nitrate as well as conductivity and filt P best explained the separation between sites in summer 2017 and autumn 2017 respectively.

5.3.2.2 Correlation between macroinvertebrates and water quality parameters between four times at each site in Florentine and Russell Falls

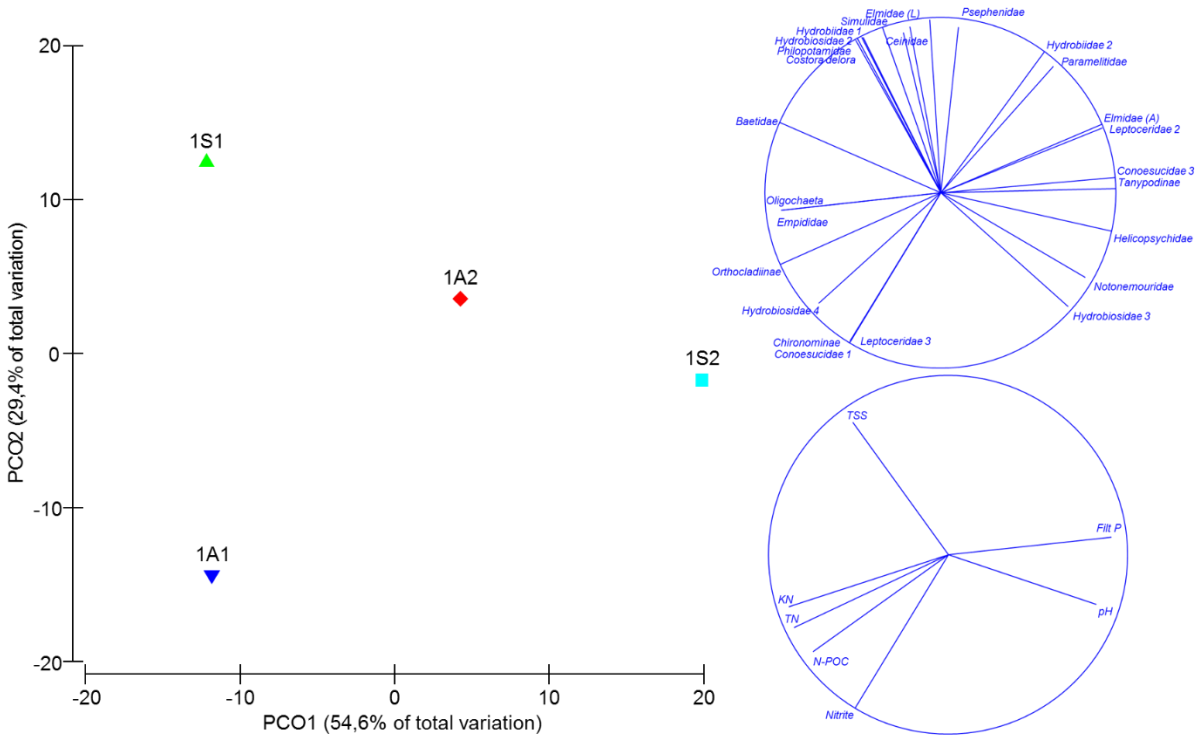
Macroinvertebrate communities at each site differed among seasons (Figure 5.5). Moreover, there was associations between macroinvertebrates and water quality parameters; which was associated with time. At the upstream site of the Florentine (1); high TSS was associated with the presence of *Costora delora,* Philopotamidae, Hydrobiosidae 2, Hydrobiidae 1, Simulidae (black fly larvae) and Ceinidae whereas nitrite was high in autumn 2016 and associated with Chironominae, Hydrobiosidae 4, Conoesucidae 1 and Leptoceridae 3. In summer 2017, Conoesucidae 3, Tanypodinae and Helicopsychidae had high abundance and were correlated with high filt P and pH. Furthermore, higher KN, TN and N-POC in year of 2016 than that of 2017 were recorded while the only variable which the explained community patterns at the upstream site of the Florentine (1) was TN.

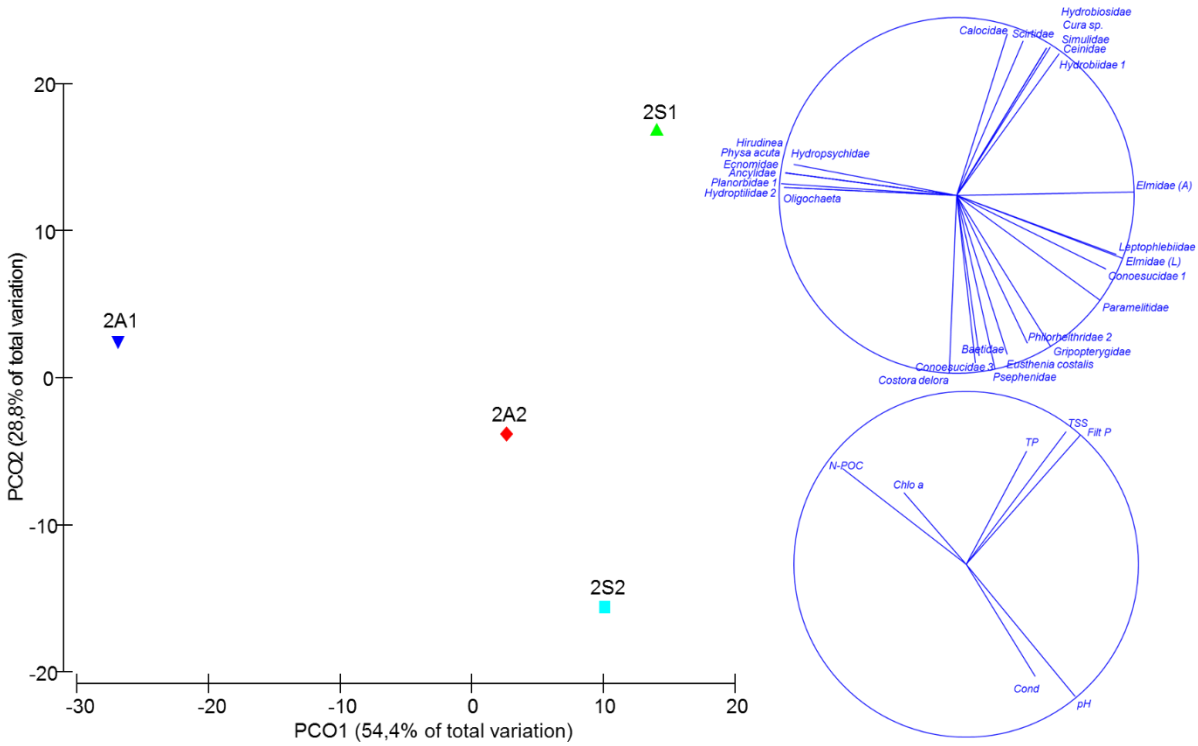
At the downstream site of the Florentine (2); the presence of *Cura sp.*, Simulidae, Ceinidae, Hydrobiosidae and Hydrobiidae 1 associated with high levels of filt P, TP and TSS in summer 2016 while high pH and conductivity were indicative of the presence of Paramelitidae, Philorheithridae 2 and Gripopterygidae in summer 2017. High levels of N-POC and Chlorophill-

a were associated of Oligochaeta, Hirudinae (leeches), *Physa acuta*, Ecnomidae, Ancylidae, Planorbidae 1, Hydropsychidae and Hydroptilidae in autumn 2016. Within those variables, filt P and N-POC were the two variables which the best correlated with the community pattern.

At the upstream site of Russell Fall (3); in autumn 2016, a higher pH was associated with the presence of Sisyridae (spongefly larvae), *Atalophlebia australis,* Tasimidae, Hydrobiosidae and *Costora delora*. In summer 2017; KN, TN and ammonia&ammonium were high, and associated with Empididae, Scimyzidae and Phreatoicidae (freshwater isopods) while conductivity and high filt P were associated with Hydrobiosidae 1&4, Psephenidae, Hydrobiosidae 1, Leptoceridae 7 and Baetidae in autumn 2017.

A correlation between invertebrates and water quality was seen in summer 2016 at the downstream site of Russell Falls (4). Specifically, high KN and TN were correlated with the presence of Hydrobiidae 2, Philorheithridae 2, Chironominae and *Lingora sp*. Furthermore, BioEnv showed that nitrite, TN and Chlorophyll-a best explained the pattern of community at Russell Fall 1 while TSS, TN and KN were the best correlated with the separation at Russell Fall 2.


1S1
1A2
1S2
1A1
PCO2 (29,4% of total variation)
PCO1 (54,6% of total variation)
Elmidae (L)
Psephenidae
Simuliidae
Hydrobiidae 1
Hydrobiosidae 2
Ceinidae
Hydrobiidae 2
Philopotamidae
Costora delora
Paramelitidae
Baetidae
Elmidae (A)
Leptoceridae 2
Conoesucidae 3
Tanypodinae
Oligochaeta
Empididae
Helicopsychidae
Orthocladiinae
Notonemouridae
Hydrobiosidae 4
Hydrobiosidae 3
Chironominae
Leptoceridae 3
Conoesucidae 1
TSS
Filt P
KN
pH
TN
N-POC
Nitrite


2S1
2A1
2A2
2S2
PCO2 (28,8% of total variation)
PCO1 (54,4% of total variation)
Hydrobiosidae
Cura sp.
Calocidae
Scirtidae
Simuliidae
Ceinidae
Hydrobiidae 1
Hirudinea
Physa acuta
Hydropsychidae
Ecnomidae
Ancylidae
Planorbidae 1
Hydroptilidae 2
Oligochaeta
Elmidae (A)
Leptophlebiidae
Elmidae (L)
Conoesucidae 1
Paramelitidae
Phlorbeithridae 2
Baetidae
Gripopterygidae
Conoesucidae 3
Eustheniа costalis
Costora delora
Psephenidae
TSS
Filt P
TP
N-POC
Chlo a
Cond
pH

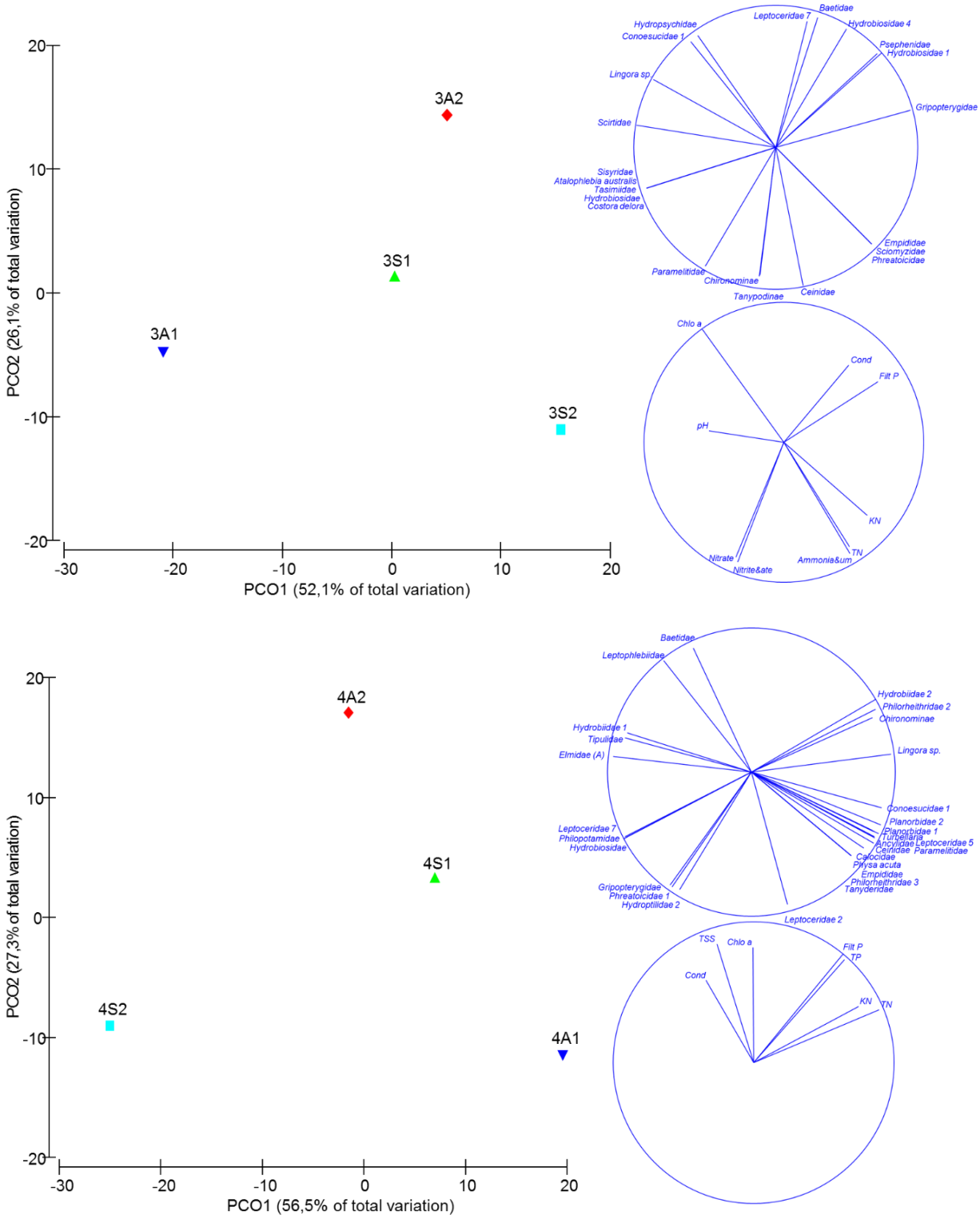


Figure 5.5: The two dimensional PCO plot showing relationship between seasons at each site in Florentine and Russell Falls (1: upstream of farm at the Florentine, 2: right at farm outlet at the Florentine, 3: upstream of farm in Russell Falls, 4: closest to farm outlet at Russell Falls. Vector overlays show both the dominant macroinvertebrate taxa for each plot and the key water quality (WQ) parameters driving the community separations (note the WQ parameters are restricted to those having vectors longer than 0.5) (S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017)

5.4 Discussion

The study illustrated that there was a clear difference in macroinvertebrate communities between rivers in Tasmania, and that these differences likely reflect both changes in prevailing habitat surrounding the river, river substrate and the effect of pollutants. These results were similar to the findings from chapter 2 and chapter 3. In particular, the two upland rivers (the Broad and the upstream site of the Florentine) surrounded by forest had a similar community structure which indicated good water quality while the lowland rivers (the Styx and the Tyenna End) surrounded by grazing and agriculture had a different community structure. The downstream site of the Florentine (impacted site) had more similar community composition to the Styx and the Tyenna End than other sites. The two small and shallow lowland rivers (the Dee and the Ouse) with high levels of anthropogenic impacts (grazing, agriculture, urbanised and industrial areas) surrounding them had significantly different invertebrate community structure from all other sites. However, it was not possible to directly relate the condition assessment provided by the species composition data to specific water quality measures.

The overriding effect of natural habitat on both the ecology and water chemistry of river systems is well known. Berkman et al. (1986) and Azrina et al. (2006) concluded that, in the absence of disturbance, differences in habitat were the main influence on invertebrate community composition. Many studies have also shown that stream water quality reflects habitat condition, and changes in land-use (Munyika et al., 2014) or ecological integrity (Li et al., 2010), and that this in turn is reflected in the invertebrate community structure. In the present study, the benthic communities clearly indicated changes in benthic condition with

more pollution-tolerant taxa and a higher nutrient load at impacted sites (the Styx, the Tyenna End, the Dee, the Ouse, the downstream of Florentine and Russell Falls) compared to cleaner sites (the Broad, the upstream of the Florentine and Russell Falls). The water quality measures on the whole remained quite good compared to the default low – risk trigger values for slightly disturbed ecosystems in Tasmania (Table 5.1) and water quality standards for farm effluents (Table 5.2), except those near aquaculture outfalls, and generally suggested better environmental condition levels than the fauna indicated. The findings from Damanik-Ambarita et al. (2016) explained that good water quality conditions tended to be correlated with a high flow velocity, less disturbance and a low conductivity; such conditions are usually found in highland rivers in mountain areas and this is consistent with the results for the Broad and the upstream site of Florentine in this study. In contrast, the sites close to the aquaculture facilities (the downstream site of Florentine and Russell Falls, and Tyenna End (the further far downstream)) all had elevated organic and inorganic matter loadings, which is likely due to waste from the farms such as unconsumed feed, excreta, and chemicals (Ackefors and Enell, 1990; Ackefors and Enell, 1994) and reducing water quality. However, some of the non-aquaculture sites, including the Dee, the Ouse and the Styx also had elevated nutrient levels, suggesting there may be other land-based sources of enrichment at these locations. Nutrient loads can rise due to manure utilisation and chemical fertilisers at agriculture sites (Damanik-Ambarita et al., 2016); which may explain the higher nutrient levels at the Dee and Ouse in this study

Table 5.1: Default low – risk trigger values for slightly disturbed ecosystems in Tasmania (Anzecc, 2000) as well as water quality standards for farm effluents (Boyd, 2003a)

Water quality parameters	Lowland River (below 150m)	Upland River (above 150m)	Farm effluents
Turbidity (NTU)	6 – 50	2 – 25	
Conductivity (mS/cm)	125 – 2000	30 – 350	
pH	6.5 – 8.0	6.5 – 7.5	6.0 – 9.0
DO (% saturation)	85 – 100	90 – 110	
Total nitrogen (mg/l)	0.500	0.480	≤ 5
Nitrate & Nitrite: NO_x – N (mg/l)	0.190	0.190	
Ammonium: NH_4^+ - N (mg/l)	0.020	0.013	
Total phosphorus (mg/l)	0.050	0.013	≤ 0.5
Dissolved reactive phosphorus (mg/l)	0.020	0.005	
Total suspended solids (mg/l)			≤ 50
5-Day biochemical oxygen demand (mg/l)			≤ 30
Dissolved oxygen (mg/l)			≥ 5

Interestingly, organic and inorganic matter levels were generally higher in summer 2016 than in autumn 2016, but higher in autumn 2017 than in summer 2017 for the impacted sites (downstream site of the Florentine, the Tyenna End, the Styx, the Dee and the Ouse). This might be a natural seasonal effect, perhaps a result of lower river flow in summer compared to autumn in 2016 where precipitation is generally higher, although the opposite results for water chemistry in 2017 were likely the result of the major flood which swept away residual nutrients and improved stream water quality. A previous study from mainland Australia showed that the impacts of nutrients, DO and low flows on river ecosystems (water quality and macroinvertebrates) were greater in drought years compared with wet years (Parr and Mason (2003). In the larger rivers (the Florentine, the Broad, the Styx, the Russell Falls and

the Tyenna End) this response was not apparent. In these systems, the water quality was generally better overall, but seemed to be cleaner in 2017 than in 2016, indicating a level of inter-annual difference. Similarly, the macroinvertebrate response in these larger river systems also corresponded to the water quality assessment, as in this case pollution tolerant taxa were more abundant in summer and autumn 2016 than in summer 2017, suggesting the river was cleaner in 2017 than in 2016. Macroinvertebrates respond to changing environmental conditions, but unlike water quality measures which give a snapshot of the condition at a specific point in time, the benthic community will reflect the integrated effect of changes in the ecosystem over time and hence are often considered a more accurate and integrated measure of environmental condition (Bennison et al., 1989; Goodnight, 1973; Slooff, 1983). In 2016 there were major floods in all these river systems, which would potentially have swept away any residual nutrients, effectively resetting the systems and resulting in improved water quality and lower numbers of pollution-tolerant taxa (Oligochaeta, Hirudinae, Physa acuta, Cura sp., Planorbidae, Simulidae, Chironominae, Orthocladiinae, Tanypodinae, Tipulidae, Hydropsychidae). It is however worth noting that at the sites adjacent to the aquaculture outfalls (downstream of Russell fall and Florentine, the Tyenna End, the Styx) the pollutant indicator taxa were still dominant after the flood. Interestingly, there was not much change in the water quality or the numbers of pollution-tolerant taxa at the smaller rivers (the Dee and the Ouse) with very low water level and current which seemed less affected by the floods; these rivers remained more stable and continued to and indicate the same temporal pattern of impact.

Overall the results suggest that the Broad, the Florentine 1 and the Russell Falls 1 might be considered clean sites; these rivers had strong flows and are located in non-agricultural,

forested locations with less impact from pollutants. The community characterising these sites comprised: Psephenidae, Baetidae, *Eusthenia costalis*, Gripopterygidae, *Atalophlebia australis*, *Costora Delora*, *Lingora sp.* and Scirtidae. These taxa have previously been shown to be found in good quality water (Chessman, 2003b; Crance, 1996; Gooderham and Tsyrlin, 2002; Goodnight, 1973). In contrast, the nominally impacted sites (the Styx, the Tyenna End, the Dee, the Ouse, the downstream of Florentine and Russell Falls) all had higher organic and inorganic matter loads, and conductivity, filtered P, KN, Nitrate, NO_x – N, Nitrite, TN, ammoniacal nitrogen (ammonia & ammonium), N-POC, TP, TSS, turbidity and Chlorophyll-a were all elevated. At these sites, pollution tolerant taxa (Gooderham and Tsyrlin, 2002; Goodnight, 1973) dominated i.e. Oligochaeta, Hirudinae, *Physa acuta*, *Cura sp.*, Planorbidae, Simulidae, Chironominae, Orthocladiinae, Tanypodinae, Tipulidae, Hydropsychidae, Phreatoicidae, Tasimiidae, Ceinidae, Caenidae, Hydrobiosidae, Leptoceridae, *Atyidae paratya*, Ecnomidae, and Sciomyzidae. Additionally, some invertebrates such as dipterans (true fly larvae), Chironomidae (midge larvae)(Ogbeibu and Oribhabor, 2002), cranefly larvae, dragonfly larvae, crayfish, filtering caddisfly, blackfly larvae, and dobsonfly larvae can be present in a wide range of water quality conditions (Crance, 1996)

The impact classification of the rivers appears to be most accurate when based on invertebrate community structure, with a number of key pollution intolerant or pollution tolerant taxa being integral to that separation. There were water quality indices correlated with the groups of taxa which best explained the separation of community pattern, but these were not categorical. There were a number of taxa (Conoesucidae, Philopotamidae, Hydroptilidae, Philorheithridae, Hydrobiidae, Paramelitidae, Leptoceridae, Helicopsychidae, Calamoceratidae, Hydropsychidae, Chironomidae) that could be present either at impacted

sites or cleaner sites and as such, whilst informative of community function, could not be considered reliable indicators. Within the pollution tolerant taxa there were also site specific differences, for example Oligochaeta, Hirudinae, *Physa acuta*, *Cura sp.*, and Planorbidae dominated at downstream sites of the Florentine and the Russell Falls whilst Tipulidae, Ceinidae, Paramelitidae, Caenidae, Hydrobiosidae, Ecnomidae, Sciomyzidae, Hydroptilidae, and Calamoceratidae had higher abundance at the Dee and the Ouse. Pollution tolerant taxa were abundant where nutrient loads were high but could still be found in cleaner conditions, and hence it is not just presence of these species that indicates impact but also their abundance. In contrast, pollution intolerant taxa were generally absent where nutrient loads were elevated but might only present at very low levels otherwise – hence these species would make good presence/ absence indicators. The findings of this study are similar to previous studies, but some of them showed specific taxa indicative of specific pollutant. Aquatic worms (Oligochaeta), family Tubificidae, freshwater leeches (class Hirudinea), and larvae and pupa of midges (Chironomidae) are strong indicators of organic pollution (Chessman, 2003a; Gooderham and Tsyrlin, 2002; Goodnight, 1973). This assumption is supported by the research of Varnosfaderany et al. (2010) which confirmed there was no Plecoptera (stonefly), Ephemeroptera (mayfly) and Trichoptera (caddisfly) at disturbed sites. Other studies suggested Trichopteran (caddisflies) (Azrina et al., 2006) and Ephemeraptera (mayflies) (Azrina et al., 2006; Lenat and Crawford, 1994b; Ogbeibu and Oribhabor, 2002); and Oligochaeta (Azrina et al., 2006; Lenat and Crawford, 1994b; Ogbeibu and Oribhabor, 2002; Slepukhina, 1984) were indicative of clean and polluted aquatic environment respectively. Lenat and Crawford (1994b) also emphasised Chironomidae indicated agricultural sites with fair water quality. For instance, Whitehurst (1991) found that the ratio of *Gammarus* (Amphipoda) : *Asellus* (Isopoda) present in a stream can be indicative of organic

pollution whereas the presence of mayfly larvae (*Baetis rhodani* and *B.vernus*) can be representative of heavy metal pollution in streams (Fialkowski et al., 2003). Many taxa of snails (e.g. *Physa spp.*) are generally present in septic streams while freshwater bivalve molluscs (Sphaeriidae) are indicators of low oxygen condition. Because water quality will fluctuate in response to daily husbandry activities such as feeding and cleaning (Kaushik and Cowey, 1991) measuring water quality to monitor river health may be most appropriate if short-term fluctuations are important. In contrast, macroinvertebrates may better show the long-term effects of any environmental changes (Goodnight, 1973) and can be indicate longer term or specific aquatic conditions (Cook, 1976). Crance (1996) and Iliopoulou-Georgudaki et al. (2003) also suggested that macroinvertebrates can be reliable indicators to monitor the health of streams. Thus, the presence of macroinvertebrates indicative of specific pollution factors can be a robust and cost-effective tool to monitor river health compared to water quality that might need laboratory analyses with higher cost. These findings highlight that not all the sampled rivers had the same characteristics; however, the response of macroinvertebrates to pollutant factors were similar. This strengthens the utility of macroinvertebrates in monitoring of impacts. Specific taxa might actually be the best (quick and most cost-effective) tool for a monitoring program e.g. where identification of one animal can provide the same assessment as several chemistry assessments or a larger community-level faunal assessment. Therefore, one macroinvertebrate taxon that is reliable and easy to identify, and that could be used on all (or particular) rivers for management decision as well as improving monitoring is necessary for effective programs.

5.5 Conclusion

In conclusion, there was a relationship between macroinvertebrates and water quality parameters. Upstream of aquaculture inputs and at undisturbed sites the water quality was consistently good and the invertebrate taxa were indictive of cleaner conditions compared to impacted sites. The less disturbed sites had a higher abundance of pollution intolerant taxa (notably group 1: Psephenidae, Baetidae, *Eusthenia costalis*, Gripopterygidae, *Atalophlebia australis*, *Costora Delora*, *Lingora sp.* and Scirtidae) which correlated with DO and pH levels greater than 9 mg/l and 7 respectively and indicated good water quality. Those clean-water taxa decreased in abundance at the impacted sites. While water quality showed higher organic and inorganic matter (conductivity, filtered P, KN, nitrate, nitrite & nitrate, nitrite, TN, ammonia & ammonium, N-POC, TP, TSS, turbidity and Chlorophyll-a) at both sites impacted by aquaculture, agriculture and grazing which were not distinguishable; the presence of certain invertebrate taxa could be indicative of specific types of pollutants. In particular, aquaculture polluted sites had a higher abundance of Oligochaeta, Planorbidae, *Physa acuta*, Hirudinae, *Cura sp.* (group 2) whereas Tipulidae, Ceinidae, Paramelitidae, Caenidae, Hydrobiosidae, Ecnomidae, Sciomyzidae, Hydroptilidae, and Calamoceratidae (group3) were indicators of agriculture and grazing polluted sites; which were high in nitrogen and phosphorus. The presence of those three certain invertebrate groups may be indicators for clean sites, aquaculture impacted sites and agriculture impacted sites respectively. Therefore, those groups of macroinvertebrates could be considered as quick, robust and cost-effective tools for aquaculture farms and government agencies wishing to assess and monitor aquatic systems. Nevertheless, further research needs to be undertaken to attempt to identify one macroinvertebrate taxon which provides the same information as groups of indicators to highlight conditions of a wide range of different river systems.

6 Chapter 6: General discussion

The comparison among rivers not impacted by aquaculture farms indicated strong differences in macroinvertebrate communities depending on river geomorphology, surrounding habitat of the catchment and pollutants from factors such as grazing, agriculture, urbanisation and industry (Chapter 2). Generally in both regions (North and South), upland rivers surrounded by forest had a similar community structure dominated by Scirtidae, Leptophlebiidae, *Eusthenia costalis*, and Elmidae which indicated good water quality; lowland rivers surrounded by grazing and agriculture had a different community structure to upland rivers (but were similar to each other) dominated by Chironomidae, Hirudinae, Planorbidae, Physidae, *Cura sp.*, Ceinidae, Paramelitidae and Oligochaeta which suggested they were mildly polluted; while small and shallow lowland rivers surrounded by high levels of anthropogenic impacts (grazing, agriculture, urbanisation and industry) had different macroinvertebrate communities from all other rivers and were dominated by Chironomidae, Hirudinae, Planorbidae, Physidae, *Cura sp.*, Ceinidae and Paramelitidae and Oligochaeta (Chapter 2). These findings highlight the large spatial variation in river macroinvertebrate communities (Feld and Hering, 2007) and the importance of both natural and anthropogenic factors in driving those patterns (Azrina et al., 2006; Usseglio-Polatera and Beisel, 2002). This variation in communities among rivers suggests establishing a baseline to determine the impacts of a salmon hatchery is important as rivers have variable water quality with some already impacted by several possible sources.

The comparison of macroinvertebrate communities at sites with vs. without aquaculture farms showed strong differences between those sites (Chapter 3) indicating effluents

originating from farms play an important role in determining macroinvertebrate composition. The sites impacted by aquaculture farm effluent (downstream site of the outfall) all had a similar macroinvertebrate community structure and were dominated by aquatic worms (Oligochaeta), fresh water snails (*Physa acuta*, Planorbidae), flat worms (*Cura sp.*, Turbellaria) and leeches (Hirudinea, Glossiphoniidae) which are pollution tolerant taxa (Gooderham and Tsyrlin, 2002)(Chapter 3, Chapter 4). Sites not impacted by aquaculture farms (upstream sites of the outfalls) were generally similar to one another except for one of the sites (the upstream site of outfall at Brumbys) (Chapter 3). The only similar study I am aware of examining the impacts of salmonid hatcheries on macroinvertebrates in rivers in Australia (Rainbow trout, Webb (2012b), determined that greater production intensity of farms resulted in stronger impacts on macroinvertebrate communities indicating larger farms are likely to cause a greater impact on receiving water. Since the time of sampling, the four salmon hatcheries monitored in this study have expanded their use of recirculating aquaculture systems (RAS) and modified other strategies (e.g. feeding) to reduce effluent released into settlement ponds and ultimately to the rivers. The hatcheries at St Patricks and Russell Falls had less impact on the streams than the hatcheries at Brumbys and Florentine but unfortunately.

The hatchery on the Florentine site produced c. 240 tonne Atlantic salmon on-growing approximately 2-2.6 million fry (sourced from Wayatinah) to smolt (range 100-150 g; average 120-130g) across the year focusing specifically on photo-manipulated production of out-of-season smolts and marine pre-smolts. Out-of-season smolt (c. 40% total) were grown on site between November and March while marine pre-smolt (c. 60% total) were produced between April and October. The farm employs both RAS and flow-through tank systems (all photo-controlled). Solid and nutrient wastes are removed via RAS management and via the

settlement pond before effluent water exits through the outfall into the Florentine River. Petuna operates the hatchery on Brumbys Ck in northern Tasmania, combining RAS tank technology with flow through tanks and raceway systems to produce c.450 tonne fish at the time of this study. Two RAS systems support egg/fry production and smolt production. In 2016-17 the site annually grew c. 2.5 million Atlantic salmon smolt (average 150g), 0.3 million two year old rainbow trout fingerlings (average 300g) and c. 10-15,000 broodstock (5,000 x 5 kg and 5-10,000 x <2 kg developing brood). The FCR achieved was 1.1:1 and approximately 20t/week (1000 t/year) of solid waste was removed via the solids filtration of the RAS systems before water exited the farm via a settlement pond. In this study, outfall and downstream 1 (downstream next to the outfall) at Brumbys were more degraded than at Florentine (Chapter 3, 4); which was consistent with the higher production at Brumbys than at Florentine, similar to the finding of Webb (2012b). Moreover, the abundance of those pollution tolerant taxa was highest at the farm outfalls but decreased gradually with distance from the outfalls, indicating a declining level of impact and recovery of macroinvertebrate communities moving downstream. This was likely due to waste matter from aquaculture farms increasing nutrients at the farm outfalls which supplies food for those taxa. However, macroinvertebrate assemblages did not fully return into the upstream condition within 800 metres downstream of the outfall. To my knowledge, there have been no previous studies in Australia of the gradient of impact downstream from aquaculture outfalls. Webb (2012b) compared one station upstream and one station downstream at five trout farm in Victoria, Australia to investigate their impacts. He detected small impacts largely dependent on farm production by employing SIGNAL, Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness and non-metric multidimensional scaling. The paucity of studies examining the impacts of aquaculture farms on rivers in Australia hinders the ability to generalise about their impacts.

6.1 Monitoring tool

The four research chapters (2 to 5), illustrated that macroinvertebrates can indicate impacts of forestation, agriculture, grazing and aquaculture on streams with communities representative of healthy habitat, mild and severe pollution. The comparison of water chemistry with macroinvertebrate communities in chapter 5 indicated macroinvertebrates can be a reliable and cost-effective tool for monitoring impacts on streams as they integrate long-term changes in water quality (smoothing short-term variability) and provided evidence for differential impacts of agriculture, grazing and aquaculture. Sites separated into clean (forestation), mild or moderate (agriculture, grazing) or severe pollution (aquaculture) by macroinvertebrates could not be distinguished by water quality parameters (nitrogen and phosphorus) alone (Chapter 5). Furthermore, water quality will fluctuate in response to daily husbandry activities such as feeding and cleaning (Kaushik and Cowey, 1991) whilst macroinvertebrates will not fluctuate on those time frames as they reflect the long-term (months-to-years) impacts of fluctuating feeds, organic matter, chemicals, and other inputs from aquaculture farms (Crance, 1996; Iliopoulou-Georgudaki et al., 2003). In terms of monitoring cost, laboratory analysis of water quality parameters likely has a much higher cost than sampling and describing macroinvertebrates which is relatively quick and cost-effective but only if indicator species can infer stream quality. Moreover, macroinvertebrate analysis can be performed by aquaculture technicians or managers using photographs of indicator species; which will help farms take the initiative in regular management and monitoring of farm effluents. Such an approach on-farm is not unusual in Tasmania where technicians on marine farms use photographic images to identify toxic and troublesome micro-algae to manage farm sites.

6.2 Indicator species

In terms of indicator species, chapter 2 and 4 suggested that indicators were characterised by location, but not by time (season). Analyses of vector loadings on the PCO plots could not be run at some sites at all sampling times. This might be because there were no significant differences in abundance of each taxa in the community at the sites across time. Therefore, we manually looked back the data, which showed that there was no consistent absence of one species in one season at all sites. This explained that indicator species were not correlated with time (seasonal changes), their presence or absence were mainly based on feeds, organic matter, chemicals, various pollutants (Crance, 1996; Iliopoulou-Georgudaki et al., 2003). Results from the four research chapters suggested that different taxa were good indicators for good water quality, polluted water and, more specifically, farm impacted water. For example, Psephenidae (water-penny beetles), Baetidae (mayflies), Scirtidae (marsh beetles), Hydrobiosidae (caseless caddis flies), Leptophlebiidae (mayflies), *Eusthenia costalis*, Gripopterygidae (stone flies), *Atalophlebia australis* (mayflies), *Costora Delora (caddis fly larvae)*, *Lingora sp.* (caddis flies) and Elmidae (riffle beetles) were indicative of good water quality. Previously, mayflies, stoneflies and caddisflies have been described as indicative of good water quality (Loch et al. (1996b) and decline at the outfall and recover downstream of trout farm effluent (Selong and Helfrich (1998). In contrast, Oligochaeta, Hirudinae, Planorbidae, *Physa acuta*, *Cura sp.*, Ceinidae and Paramelitidae, Chironomidae, Orthocladiinae, Tanyppodina, Sphaeriidae, Tipulidae, Ecnomidae, Sciomyzidae, Hydroptilidae, Hydrobiosiidae and Calamoceratidae were indicative of pollution (agriculture, grazing and aquaculture). Within those 'pollution' taxa, Oligochaeta, Planorbidae, *Physa acuta,* Hirudinae, *Cura sp.* were all good indicators of the farm impacts (chapter 3 and 4) while Tipulidae, Ceinidae, Paramelitidae, Caenidae, Hydrobiosidae, Ecnomidae, Sciomyzidae,

Hydroptilidae, and Calamoceratidae were indicators of agriculture and grazing polluted sites (chapter 2 and 5).

Identification of one indicator species or taxa that can provide the same assessment as the overall community or water quality parameters would make monitoring much simpler and easier to perform. Thus, stream water quality conditions could be indicated by differences in abundance of one indicator species. Here, three taxa appeared useful as indicator species: Baetidae, Hydropsychidae and Oligochaeta which were all common and large enough to be seen by the naked-eye. Baetidae were abundant at cleaner sites and decreased at impacted sites while Hydropsychidae dominated at polluted sites but were less abundant at cleaner sites. However, both taxa could not consistently indicate specific pollution conditions. In particular, a higher number of Baetidae at some sites showed more impacted conditions instead of more cleaner condition than lower number of Baetidae. Similarly, fewer Hydropsychidae highlighted more impacted water quality. In contrast, Oligochaeta were highly abundant at the farm outfalls (chapter 2, 3 and 4) and appeared to differentiate impacted from clean sites as well as downstream from upstream sites. The increasing level of impacts with an increase in Oligochaeta abundance (absent at healthy sites, absent or present in very low abundance at clean sites, present at mild impacted sites; and high abundance at moderate and severe impacted sites) suggests they will be a good indicator or monitoring tool (Brinkhurst, 1966; Chapman et al., 1982; Slepukhina, 1984; Uzunov et al., 1988).

6.3 Management implication

In the four chapters; total abundance, taxa richness, Simpson diversity index, SIGNAL 2, PERMANOVA and PCO were employed to determine changes in macroinvertebrate communities between sites and stations. While total abundance, taxa richness and diversity

illustrated general information; they could not determine clear differences in macroinvertebrate communities within regions and between sites over seasons. PERMANOVA and SIGNAL 2 could clearly distinguish community and water quality differences between sites (chapter 5). However, those methods require a reasonably high technical knowledge (being able to identify many macroinvertebrate taxa) and statistical proficiency (multivariate analysis). Indicator species, specifically Oligochaeta, appear to be a quick, simple and cost-effective tool for farms wishing to monitor, manage and minimise the impacts of waste discharge on rivers. Oligochaeta can detect impacts and counting them is much less time consuming than undertaking PCO and SIGNAL 2. Generally, the higher the organic matter in the bottom substrate, the more suitable the environment is for Oligochaeta and they have a higher abundance (Uzunov et al., 1988). In this study, the absence of Oligochaeta suggested healthy stream conditions, 1 – 50 individuals indicated mild pollution, 50 - 900 individuals indicated moderate pollution while a very high abundance (> 1000 individuals) indicated pollution or aquaculture impacted conditions at farm discharge points. Furthermore, Oligochaeta can be easily identified by aquaculture technicians for assessment. Oligochaeta have been used globally as an effective indicator of impact (Brinkhurst, 1966; Chapman et al., 1982; Slepukhina, 1984; Uzunov et al., 1988).

These findings provide mixed support for current regulatory requirements. EPA requires sampling in autumn and spring to assess and monitor water chemistry, macroinvertebrates and algae at downstream site of the outfall. The similarities in macroinvertebrate communities in summer and autumn as well as in winter and spring (chapter 4) supports the EPA requirements and should be adequate to take into account seasonal differences. In contrast, live picking and identification with the naked eye of the first 100 invertebrates

(AUSRIVAS method) for analysis from the EPA might not detect some important taxa which may indicate impacts. A previous study in Australia, Webb (2012b), also employed live picking with the naked eye, but picked for 30 minutes to detect impacts. This suggests that live picking would be appropriate method but picking 100 animal or picking for 30 minutes would raise a question about the accuracy of results as the method may lose some important taxa behind. Therefore, live picking with the naked eye of single indicator species may be feasible as it can be quick and simple as well as being indicative of the whole community. The EPA sampling of upstream and downstream of the farms limits the examination of downstream recovery progress identified in this study. I would recommend using Oligochaeta as an indicator species over time and over a range of downstream stations to inform impact and recovery of streams.

6.4 Conclusion

This study has increased our understanding of macroinvertebrate communities in different rivers, the impacts of aquaculture farms on receiving waters and the recovery processes associated with organic enrichment from farm effluents. The evaluation of macroinvertebrate response to changes of source of impacts suggests the finding of the study using a single taxa instead of the whole community composition, Oligochaeta, as indicator species, is a simple, quick and reliable method for monitoring impacts and recovery of macroinvertebrates in rivers. Nevertheless, such monitoring tools must be carefully evaluated to ensure that deterioration by the build-up of waste discharge is not happening.

References

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. BioScience. 39, 378-286.

Aber, J.D., Melillo, J.M., Nadelhoffer, K.J., Pastor, J., Boone, R.D., 1991. Factors controlling nitrogen cycling and nitrogen saturation in northern temperate forest ecosystems. Ecological applications. 1, 303-315.

Ackefors, H., Enell, M., 1990. Discharge of nutrients from Swedish fish farming to adjacent sea areas. AMBIO, 28-35.

Ackefors, H., Enell, M., 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. Journal of Applied Ichthyology. 10, 225-241.

Allan, J.D., Erickson, D.L., Fay, J., 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater biology. 37, 149-161.

Amirkolaie, A.K., 2008. Environmental impact of nutrient discharged by aquaculture waste water on the Haraz River. Journal of Fisheries and Aquatic Sciences. 3, 275-279.

Amirkolaie, A.K., 2011. Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. Reviews in Aquaculture. 3, 19-26.

Anderson, M., Gorley, R., Clarke, K., 2008. Plymouth: Primer-E. PERMANOVA+ for PRIMER: Guide to software and statistical methods.

Anzecc, A., 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, 1-103.

Armitage, P., 1978. Downstream changes in the composition, numbers and biomass of bottom fauna in the Tees below Cow Green Reservoir and in an unregulated tributary Maize Beck, in the first five years after impoundment. Hydrobiologia. 58, 145-156.

Armitage, P., Moss, D., Wright, J., Furse, M., 1983a. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water research. 17, 333-347.

Armitage, P.D., Moss, D., Wright, J.F., Furse, M.T., 1983b. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water Research. 17, 333-347.

Ashton, P.H., Richardson, B.J., 1995. Biological monitoring of the marine ocean outfall at black rock, Victoria, Australia. Marine Pollution Bulletin. 31, 334-340.

Aure, J., Stigebrandt, A., 1990. Quantitative estimates of the eutrophication effects of fish farming on fjords. Aquaculture. 90, 135-156.

AUSRIVAS, 2014. AUSRIVAS Bioassessment: Macroinvertebrate. Australian River Assessment System, Australia.

Azrina, M.Z., Yap, C.K., Rahim Ismail, A., Ismail, A., Tan, S.G., 2006. Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. Ecotoxicology and Environmental Safety. 64, 337-347.

Balderas, E.C.S., Grac, C., Berti-Equille, L., Hernandez, M.A.A., 2016. Potential application of macroinvertebrates indices in bioassessment of Mexican streams. Ecological Indicators. 61, 558-567.

Barbour, M.T., Gerritsen, J., Snyder, B., Stribling, J., 1999. Rapid bioassessment protocols for use in streams and wadeable rivers. USEPA, Washington.

Barnes, J.B., Vaughan, I.P., Ormerod, S.J., 2013. Reappraising the effects of habitat structure on river macroinvertebrates. Freshwater Biology. 58, 2154-2167.

Barrett, N.S., Buxton, C.D., Edgar, G.J., 2009. Changes in invertebrate and macroalgal populations in Tasmanian marine reserves in the decade following protection. Journal of Experimental Marine Biology and Ecology. 370, 104-119.

Beaujouan, V., Durand, P., Ruiz, L., 2001. Modelling the effect of the spatial distribution of agricultural practices on nitrogen fluxes in rural catchments. Ecological modelling. 137, 93-105.
Bennison, G., Hillman, T., Suter, P., Commission, M.-D.B., 1989. Macroinvertebrates of the River Murray: survey and monitoring, 1980-1985. Murray-Darling Basin Commission.
Bergheim, A., Selmer-Olsen, A., 1978. River pollution from a large trout farm in Norway. Aquaculture. 14, 267-270.
Bergheim, A., Sivertsen, A., 1981. Oxygen consuming properties of effluents from fish farms. Aquaculture. 22, 185-187.
Berkman, H.E., Rabeni, C.F., Boyle, T.P., 1986. Biomonitors of stream quality in agricultural areas: fish versus invertebrates. Environmental Management. 10, 413-419.
Boaventura, R., Pedro, A.M., Coimbra, J., Lencastre, E., 1997. Trout farm effluents: characterization and impact on the receiving streams. Environmental Pollution. 95, 379-387.
Bonsdorff, E., Blomqvist, E., Mattila, J., Norkko, A., 1997. Coastal eutrophication: causes, consequences and perspectives in the archipelago areas of the northern Baltic Sea. Estuarine, coastal and shelf science. 44, 63-72.
Borja, A., Miles, A., Occhipinti-Ambrogi, A., Berg, T., 2009. Current status of macroinvertebrate methods used for assessing the quality of European marine waters: implementing the Water Framework Directive. Hydrobiologia. 633, 181-196.
Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., 2010. Aquaculture: global status and trends. Philosophical Transactions of the Royal Society B: Biological Sciences. 365, 2897-2912.
Boyd, C.E., 2003a. Guidelines for aquaculture effluent management at the farm-level. Aquaculture. 226, 101-112.
Boyd, C.E., 2003b. Guidelines for aquaculture effluent management at the farm-level. Aquaculture. 226, 101-112.
Bramley, R., Roth, C., 2002. Land-use effects on water quality in an intensively managed catchment in the Australian humid tropics. Marine and Freshwater Research. 53, 931-940.
Brinkhurst, R., 1966. The Tubificidae (Oligochaeta) of polluted waters: With 3 figures in the text. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen. 16, 854-859.
Brittain, J.E., Saltveit, S.J., Castella, E., Bogen, J., Bønsnes, T.E., Blakar, I., Bremnes, T., Haug, I., Velle, G., 2001. The macroinvertebrate communities of two contrasting Norwegian glacial rivers in relation to environmental variables. Freshwater Biology. 46, 1723-1736.
Brown, C., land-based trout farms affecting streams in the south-western Cape, South Africa.
Brown, C., 1996. Macroinvertebrate community patterns in relation to physico-chemical parameters measured at two land-based trout farms affecting streams in the south-western Cape, South Africa. Archiv fur Hydrobiologie. 138, 57-76.
Brown, C., 2001. A comparison of several methods of assessing river condition using benthic macroinvertebrate assemblages. Southern African Journal of Aquatic Sciences. 26, 135-147.
Brown, C., King, J., 1995. The effects of trout-farm effluents on benthic invertebrate community structure in rivers in the south-western Cape, South Africa. Southern African Journal of Aquatic Science. 21, 3-21.
Brown, C.A., 1998. Modelling and managing the effects of trout farms on Cape Rivers. thesis. University of Cape Town.
Brown, J., Gowen, R., McLusky, D., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. Journal of Experimental Marine Biology and Ecology. 109, 39-51.
Brua, R., Culp, J., Benoy, G., 2011. Comparison of benthic macroinvertebrate communities by two methods: Kick- and U-net sampling. Hydrobiologia. 658, 293-302.

Buffagni, A., Crosa, G., Harper, D., Kemp, J., 2000. Using macroinvertebrate species assemblages to identify river channel habitat units: an application of the functional habitats concept to a large, unpolluted Italian river (River Ticino, northern Italy). Hydrobiologia. 435, 213-225.
Buschmann, A.H., López, D.A., Medina, A., 1996. A review of the environmental effects and alternative production strategies of marine aquaculture in Chile. Aquacultural Engineering. 15, 397-421.
Buss, D.F., Borges, E.L., 2008. Application of rapid bioassessment protocols (RBP) for benthic macroinvertebrates in Brazil: comparison between sampling techniques and mesh sizes. Neotropical Entomology. 37, 288-295.
Cairns Jr, J., 2017. Biological monitoring: Part VI—future needs. Biological Monitoring in Water Pollution, 941.
Callier, M.D., Richard, M., McKindsey, C.W., Archambault, P., Desrosiers, G., 2009. Responses of benthic macrofauna and biogeochemical fluxes to various levels of mussel biodeposition: An in situ “benthocosm” experiment. Marine Pollution Bulletin. 58, 1544-1553.
Camargo, J., 1993. Macrobenthic surveys as a valuable tool for assessing freshwater quality in the Iberian Peninsula. Environ Monit Assess. 24, 71-90.
Camargo, J.A., 1992a. Temporal and spatial variations in dominance, diversity and biotic indices along a limestone stream receiving a trout farm effluent. Water, Air, and Soil Pollution. 63, 343-359.
Camargo, J.A., 1992b. Structural and trophic alterations in macrobenthic communities downstream from a fish farm outlet. Hydrobiologia. 242, 41-49.
Camargo, J.A., 1994. The importance of biological monitoring for the ecological risk assessment of freshwater pollution: a case study. Environment International. 20, 229-238.
Camargo, J.A., 2019. Positive responses of benthic macroinvertebrates to spatial and temporal reductions in water pollution downstream from a trout farm outlet. Knowledge & Management of Aquatic Ecosystems, 16.
Camargo, J.A., Gonzalo, C., 2007. Physicochemical and biological changes downstream from a trout farm outlet: Comparing 1986 and 2006 sampling surveys. Limnetica. 26, 405-414.
Camargo, J.A., Alonso, Á., De la Puente, M., 2005. Eutrophication downstream from small reservoirs in mountain rivers of Central Spain. Water Research. 39, 3376-3384.
Camargo, J.A., Gonzalo, C., Alonso, Á., 2011. Assessing trout farm pollution by biological metrics and indices based on aquatic macrophytes and benthic macroinvertebrates: A case study. Ecological Indicators. 11, 911-917.
Cao, Y., Bark, A., Williams, W.P., 1996. Measuring the responses of macroinvertebrate communities to water pollution: a comparison of multivariate approaches, biotic and diversity indices. Hydrobiologia. 341, 1-19.
Capone, D.G., Weston, D.P., Miller, V., Shoemaker, C., 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture. 145, 55-75.
Carew, M., Nichols, S., Batovska, J., St Clair, R., Murphy, N., Blacket, M., Shackleton, M., 2017. A DNA barcode database of Australia’s freshwater macroinvertebrate fauna. Marine and Freshwater Research. 68, 1788-1802.
Carr, O., Goulder, R., 1990a. Fish-farm effluents in rivers—I. Effects on bacterial populations and alkaline phosphatase activity. Water Research. 24, 631-638.
Carr, O., Goulder, R., 1990b. Fish-farm effluents in rivers—II. Effects on inorganic nutrients, algae and the macrophyte Ranunculus penicillatus. Water Research. 24, 639-647.
Carroll, M.L., Cochrane, S., Fieler, R., Velvin, R., White, P., 2003a. Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. Aquaculture. 226, 165-180.

Carroll, M.L., Cochrane, S., Fieler, R., Velvin, R., White, P., 2003b. Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. Aquaculture. 226, 165-180.
Chapman, P.M., Farrell, M.A., Brinkhurst, R.O., 1982. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquatic Toxicology. 2, 47-67.
Chará-Serna, A.M., Chará, J., Giraldo, L.P., Zúñiga, M.d.C., Allan, J.D., 2015. Understanding the impacts of agriculture on Andean stream ecosystems of Colombia: a causal analysis using aquatic macroinvertebrates as indicators of biological integrity. freshwater science. 34, 727-740.
Chessman, B., McEvoy, P., 1997. Towards diagnostic biotic indices for river macroinvertebrates. Hydrobiologia. 364, 169-182.
Chessman, B., Growns, I., Currey, J., Plunkett-Cole, N., 1999. Predicting diatom communities at the genus level for the rapid biological assessment of rivers. Freshwater Biology. 41, 317-331.
Chessman, B.C., 1995. Rapid assessment of rivers using macroinvertebrates: A procedure based on habitat-specific sampling, family level identification and a biotic index. Australian Journal of Ecology. 20, 122-129.
Chessman, B.C., 2003a. SIGNAL 2. iv: A Scoring System for Macroinvertebrates (water Bugs') in Australian Rivers: User Manual. Department of the Environment and Heritage.
Chessman, B.C., 2003b. New sensitivity grades for Australian river macroinvertebrates. Marine and Freshwater Research. 54, 95-103.
Chow-Fraser, P., Lougheed, V., Le Thiec, V., Crosbie, B., Simser, L., Lord, J., 1998. Long-term response of the biotic community to fluctuating water levels and changes in water quality in Cootes Paradise Marsh, a degraded coastal wetland of Lake Ontario. Wetlands Ecology and Management. 6, 19-42.
Cirmo, C.P., McDonnell, J.J., 1997. Linking the hydrologic and biogeochemical controls of nitrogen transport in near-stream zones of temperate-forested catchments: a review. Journal of Hydrology. 199, 88-120.
Clarke, K., Gorley, R., 2006a. PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research). Plymouth: Primer-E Ltd.
Clarke, K., Gorley, R., 2006b. PRIMER v6 user manual and program. PRIMER-E Ltd: Plymouth, UK.
CLARKE, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian journal of ecology. 18, 117-143.
Clarke, R.T., Wright, J.F., Furse, M.T., 2003. RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. Ecological Modelling. 160, 219-233.
Clenaghan, C., Giller, P., O'halloran, J., Hernan, R., 1998. Stream macroinvertebrate communities in a conifer-afforested catchment in Ireland: relationships to physico-chemical and biotic factors. Freshwater Biology. 40, 175-193.
Conor Keitzer, S., Goforth, R.R., 2013. Salamander diversity alters stream macroinvertebrate community structure. Freshwater Biology. 58, 2114-2125.
Cook, S.E., 1976. Quest for an index of community structure sensitive to water pollution. Environmental Pollution (1970). 11, 269-288.
Cornel, G.E., Whoriskey, F.G., 1993. The effects of rainbow trout (Oncorhynchus mykiss) cage culture on the water quality, zooplankton, benthos and sediments of Lac du Passage, Quebec. Aquaculture. 109, 101-117.
Coste, M., Boutry, S., Tison-Rosebery, J., Delmas, F., 2009. Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). Ecological indicators. 9, 621-650.
Crance, J., 1996. Streams: a national heritage worth preserving. Alabama Cooperative Extension System, Auburn University.

Crunkilton, R.L., Duchrow, R.M., 1991. Use of stream order and biological indices to assess water quality in the Osage and Black river basins of Missouri. Hydrobiologia. 224, 155-166.
Czerniawska-Kusza, I., 2005. Comparing modified biological monitoring working party score system and several biological indices based on macroinvertebrates for water-quality assessment. Limnologica - Ecology and Management of Inland Waters. 35, 169-176.
Damanik-Ambarita, M.N., Lock, K., Boets, P., Everaert, G., Nguyen, T.H.T., Forio, M.A.E., Musonge, P.L.S., Suhareva, N., Bennetsen, E., Landuyt, D., 2016. Ecological water quality analysis of the Guayas river basin (Ecuador) based on macroinvertebrates indices. Limnologica-Ecology and Management of Inland Waters. 57, 27-59.
Daya, B., Pant, K., 2017. Biomonitoring of wetland using macrophytes and macroinvertebrates. Malaysian Journal of Sustainable Agriculture.
DE, 1999. Environment Protection and Biodiversity Conservation Act 1999. Department of the Environment, Canberra, Australia.
De Pauw, N., Gabriels, W., Goethals, P.L., 2006. River monitoring and assessment methods based on macroinvertebrates. John Wiley and Son, Ltd.: Chichester, UK.
DeNicola, D.M., Eyto, E.d., Wemaere, A., Irvine, K., 2004. USING EPILITHIC ALGAL COMMUNITIES TO ASSESS TROPHIC STATUS IN IRISH LAKES1. Journal of Phycology. 40, 481-495.
DEP, 2009. Derwent Estuary Program: Environmental Management Plan. in: Department of Primary Industries, P., Water and Environment (Ed.).
Dewson, Z.S., James, A.B., Death, R.G., 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. Journal of the North American Benthological Society. 26, 401-415.
Dora, S.L., Maiti, S., Tiwary, R., Anshumali, A., 2010. ALGAE AS AN INDICATOR OF RIVER WATER POLLUTION–A. The Bioscan Special Issue. 2, 413-422.
dos Santos Rosa, R., Aguiar, A.C.F., Boëchat, I.G., Gücker, B., 2013. Impacts of fish farm pollution on ecosystem structure and function of tropical headwater streams. Environmental pollution. 174, 204-213.
Doughty, C., McPhail, C., 1995. Monitoring the environmental impacts and consent compliance of freshwater fish farms. Aquaculture Research. 26, 557-565.
DPIPWE, 2014. Water: Water Monitoring and Assessment. Department of Primary Industries, Parks, Water and Environment, Hobart, Tasmania.
Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews. 81, 163-182.
Dumnicka, E., 2002. Upper Vistula river: response of aquatic communities to pollution and impoundment. X. Oligochaete taxocens. Polish Journal of Ecology. 50.
Dumnicka, E., Galas, J., 2002. Factors affecting the distribution of Oligochaeta in small high mountain ponds (Tatra Mts, Poland). Archiv für Hydrobiologie. 156, 121-133.
Ebeling, J.M., Timmons, M.B., 2012. Recirculating Aquaculture Systems. Aquaculture Production Systems, 245.
Ebeling, J.M., Ogden, S.R., Sibrell, P.L., Rishel, K.L., 2004. Application of chemical coagulation aids for the removal of suspended solids (TSS) and phosphorus from the microscreen effluent discharge of an intensive recirculating aquaculture system. North American Journal of Aquaculture. 66, 198-207.
Edgar, G.J., Barrett, N.S., 2000. Effects of Catchment Activities on Macrofaunal Assemblages in Tasmanian Estuaries. Estuarine, Coastal and Shelf Science. 50, 639-654.
Edgar, G.J., Barrett, N.S., 2002. Benthic macrofauna in Tasmanian estuaries: scales of distribution and relationships with environmental variables. Journal of Experimental Marine Biology and Ecology. 270, 1-24.
Edgar, G.J., Barrett, N.S., Last, P.R., 1999. The distribution of macroinvertebrates and fishes in Tasmanian estuaries. Journal of Biogeography. 26, 1169-1189.

Edgar, G.J., Macleod, C.K., Mawbey, R.B., Shields, D., 2005. Broad-scale effects of marine salmonid aquaculture on macrobenthos and the sediment environment in southeastern Tasmania. Journal of Experimental Marine Biology and Ecology. 327, 70-90.
Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J., Leese, F., 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods in Ecology and Evolution. 8, 1265-1275.
Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology letters. 10, 1135-1142.
Enell, M., 1987. Environmental impact of cage fish farming with special reference to phosphorus and nitrogen loadings. ICES Paper.
Eriksen, R., Koehnken, L., Brooks, A., Ray, D., 2011. Derwent Catchment Review. in: Department of Primary Industries, P., Water and Environment (Ed.).
Evans, R.O., Wayne Skaggs, R., Wendell Gilliam, J., 1995. Controlled versus conventional drainage effects on water quality. Journal of Irrigation and Drainage Engineering. 121, 271-276.
Extence, C., Balbi, D., Chadd, R., 1999. River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. Regulated Rivers: Research & Management. 15, 543-574.
FAO, 2018. The State of World Fisheries and Aquaculture-2018. FAO Fisheries and Aquaculture Department.
Feld, C.K., Hering, D., 2007. Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshwater Biology. 52, 1380-1399.
Fetscher, A.E., McLaughlin, K., 2008. Incorporating Bioassessment Using Freshwater Algae into California's Surface Water Ambient Monitoring Program (SWAMP). Prepared for the Technical Report. 563.
Fetscher, A.E., Busse, L., Ode, P.R., 2009. Standard operating procedures for collecting stream algae samples and associated physical habitat and chemical data for ambient bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP. 2.
Fialkowski, W., Klonowska-Olejnik, M., Smith, B.D., Rainbow, P.S., 2003. Mayfly larvae (Baetis rhodani and B. vernus) as biomonitors of trace metal pollution in streams of a catchment draining a zinc and lead mining area of Upper Silesia, Poland. Environmental Pollution. 121, 253-267.
Fierro, P., Valdovinos, C., Vargas-Chacoff, L., Bertrán, C., Arismendi, I., 2017. Macroinvertebrates and fishes as bioindicators of stream water pollution, Water Quality. Intechopen Rijeka, pp. 23-38.
FRDC, 2017. Australian Fisheries Statistics 2016. Fisheries Research and Development Corporation, Australia.

.
Fries, L.T., Bowles, D.E., 2002. Water Quality and Macroinvertebrate Community Structure Associated with a Sportfish Hatchery Outfall. North American Journal of Aquaculture. 64, 257-266.
Furlonge, T., Dyer, F., Davis, J., 2015. The influence of differing protected area status and environmental factors on the macroinvertebrate fauna of temperate austral wetlands. Global Ecology and Conservation. 4, 277-290.
Gasith, A., Resh, V.H., 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual review of ecology and systematics, 51-81.
Gilliam, J., Skaggs, R., 1986. Controlled agricultural drainage to maintain water quality. Journal of Irrigation and Drainage Engineering. 112, 254-263.

Gooderham, J., Tsyrlin, E., 2002. The waterbug book: a guide to the freshwater macroinvertebrates of temperate Australia. CSIRO publishing.
Goodnight, C.J., 1973. The Use of Aquatic Macroinvertebrates as Indicators of Stream Pollution. Transactions of the American Microscopical Society. 92, 1-13.
Gowen, R., Bradbury, N., 1987. The ecological impact of salmonid farming in coastal waters: a review. Oceanography and marine biology. 25, 563-575.
Gowen, R., Ezzi, I., Rosenthal, H., Maekinen, T., 1990. Environmental impact of aquaculture activities. European Aquaculture Society Special Publication (Belgium).
Guilpart, A., Roussel, J.-M., Aubin, J., Caquet, T., Marle, M., Le Bris, H., 2012. The use of benthic invertebrate community and water quality analyses to assess ecological consequences of fish farm effluents in rivers. Ecological Indicators. 23, 356-365.
Hall, P., Holby, O., Kollberg, S., Samuelsson, M.-O., 1992. Chemical fluxes and mass balances in a marine fish cage farm. Nitrogen. Mar. Ecol. Progr. Ser. 89: 81-91.
Hall, R.I., Leavitt, P.R., Quinlan, R., Dixit, A.S., Smol, J.P., 1999. Effects of agriculture, urbanization, and climate on water quality in the northern Great Plains. Limnology and Oceanography. 44, 739-756.
Hardie, S.A., Bobbi, C.J., Barmuta, L.A., 2012. Macroinvertebrate and water quality responses to low flows in Tasmanian rivers. National Water Commission, Canberra.
Harding, W., Archibald, C., Taylor, J., 2005. The relevance of diatoms for water quality assessment in South Africa: A position paper. Water SA. 31, p. 41-46.
Hargrave, B., 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. Mar. Ecol. Prog. Ser. 96, 249-257.
Heinen, J.M., Hankins, J.A., Adler, P.R., 1996. Water quality and waste production in a recirculating trout-culture system with feeding of a higher-energy or a lower-energy diet. Aquaculture Research. 27, 699-710.
Helfrich, L.A., Libey, G., 1991. Fish farming in recirculating aquaculture systems (RAS). Virginia State Cooperative Service.
Hirst, A., Alpine, J., Crawford, C., 2006. Benthic macroinvertebrate communities of high conservation value Thirsty and Little Thirsty Lagoons, Cape Barren Island, Tasmania. 17-24 pp.
Holmer, M., Kristensen, E., 1992. Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. Marine ecology progress series. Oldendorf. 80, 191-201.
Houle, J.E., Farnsworth, K.D., Rossberg, A.G., Reid, D.G., Hilborn, R., 2012. Assessing the sensitivity and specificity of fish community indicators to management action. Canadian Journal of Fisheries and Aquatic Sciences. 69, 1065-1079.
Humphries, P., 1996. Aquatic macrophytes, macroinvertebrate associations and water levels in a lowland Tasmanian river. Hydrobiologia. 321, 219-233.
Humphries, P., Davies, P.E., Mulcahy, M.E., 1996. Macroinvertebrate assemblages of littoral habitats in the Macquarie and Mersey rivers, Tasmania: Implications for the management of regulated rivers. Regulated Rivers: Research & Management. 12, 99-122.
Husevåg, B., Lunestad, B., Johannessen, P., Enger, Ø., Samuelsen, O., 1991. Simultaneous occurrence of Vibrio salmonicida and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. Journal of Fish Diseases. 14, 631-640.
IFS, 2013. Anual Report 2012-2013: Managing our world class fishery. Inland Fisheries Service, New Norfolk, Tasmania.
Iliopoulou-Georgudaki, J., Kantzaris, V., Katharios, P., Kaspiris, P., Georgiadis, T., Montesantou, B., 2003. An application of different bioindicators for assessing water quality: a case study in the rivers Alfeios and Pineios (Peloponnisos, Greece). Ecological indicators. 2, 345-360.
Imbert, J.B., Perry, J.A., 2000. Drift and benthic invertebrate responses to stepwise and abrupt increases in non-scouring flow. Hydrobiologia. 436, 191-208.

Ingram, B., 1999. A phosphorus model for trout farming in the Goulburn-Broken catchment. Towards best practice in landbased salmonid farming: Options for treatment, re-use and disposal of effluent, ed. BA Ingram, 26-41.
Jarvie, H., Whitton, B., Neal, C., 1998. Nitrogen and phosphorus in east coast British rivers: speciation, sources and biological significance. Science of the Total environment. 210, 79-109.
Jarvis, A.P., Younger, P.L., 2000. Broadening the scope of mine water environmental impact assessment: a UK perspective. Environmental Impact Assessment Review. 20, 85-96.
Jegatheesan, V., Zeng, C., Shu, L., Manicom, C., Steicke, C., 2007. Technological advances in aquaculture farms for minimal effluent discharge to oceans. Journal of Cleaner Production. 15, 1535-1544.
Johnson, K.S., 2007. Field and laboratory methods for using the MAIS (Macroinvertebrate Aggregated Index for Streams) in rapid bioassessment of Ohio streams. Athens: Ohio University Department of Biological Sciences.
Kaeser, A.J., Sharpe, W.E., 2006. Patterns of distribution and abundance of Tubifex tubifex and other aquatic oligochaetes in Myxobolus cerebralis enzootic areas in Pennsylvania. Journal of Aquatic Animal Health. 18, 64-78.
Kannel, P.R., Lee, S., Lee, Y.-S., Kanel, S.R., Khan, S.P., 2007. Application of water quality indices and dissolved oxygen as indicators for river water classification and urban impact assessment. Environ Monit Assess. 132, 93-110.
Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K.-N., Plaiti, W., 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES Journal of Marine Science: Journal du Conseil. 57, 1462-1471.
Kaushik, S., Cowey, C., 1991. Dietary factors affecting nitrogen excretion by fish. Nutritional strategies and aquaculture waste, 3-19.
Kelly, L., Stellwagen, J., Bergheim, A., 1996. Waste loadings from a freshwater Atlantic Salmon farm in Scotland. JAWRA Journal of the American Water Resources Association. 32, 1017-1025.
Kelly, L.A., 1993. Release rates and biological availability of phosphorus released from sediments receiving aquaculture wastes. Hydrobiologia. 253, 367-372.
Kendra, W., 1991. Quality of salmonid hatchery effluents during a summer low-flow season. Transactions of the American Fisheries Society. 120, 43-51.
Kerry, J., Hiney, M., Coyne, R., NicGabhainn, S., Gilroy, D., Cazabon, D., Smith, P., 1995. Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms. Aquaculture. 131, 101-113.
Kırkagaç, M.U., Pulatsü, S., Köksal, G., 2004. Effects Of Land-Based Trout Farms On The Benthis Macroinvertebrate Community In A Turkish Brook.
Kırkağaç, M.U., Pulatsu, S., Topcu, A., 2009. Trout Farm Effluent Effects on Water Sediment Quality and Benthos. CLEAN – Soil, Air, Water. 37, 386-391.
Koçer, M.A.T., Kanyılmaz, M., Yılayaz, A., Sevgili, H., 2013. Waste loading into a regulated stream from land-based trout farms. Aquaculture Environment Interactions. 3, 187-195.
Komulaynen, S., 2002. Use of phytoperiphyton to assess water quality in north–western Russian rivers. J Appl Phycol. 14, 57-62.
Lalonde, B.A., Garron, C., Mercier, V., 2016. Analysis of benthic invertebrate communities downstream of land-based aquaculture facilities in Nova Scotia, Canada. Cogent Environmental Science. 2, 1136099.
Lemley, D.A., Adams, J.B., Bate, G.C., 2016. A review of microalgae as indicators in South African estuaries. South African Journal of Botany. 107, 12-20.
Lenat, D., Crawford, J.K., 1994a. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. Hydrobiologia. 294, 185-199.
Lenat, D.R., Crawford, J.K., 1994b. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. Hydrobiologia. 294, 185-199.

Leuven, R.S., Brock, T.C., van Druten, H.A., 1985. Effects of preservation on dry-and ash-free dry weight biomass of some common aquatic macro-invertebrates. Hydrobiologia. 127, 151-159.
Li, L., Zheng, B., Liu, L., 2010. Biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends. Procedia environmental sciences. 2, 1510-1524.
Loch, D.D., West, J.L., Perlmutter, D.G., 1996a. The effect of trout farm effluent on the taxa richness of benthic macroinvertebrates. Aquaculture. 147, 37-55.
Loch, D.D., West, J.L., Perlmutter, D.G., 1996b. The effect of trout farm effluent on the taxa richness of benthic macroinvertebrates. Aquaculture. 147, 37-55.
Lohberger, L., 2015. Water users urged to report bad smells, taste, Mercury, Hobart.
Luttrell, A., 2015. TasWater examining reports of taste issues in Hobart's drinking water, Mercury, Hobart.
Macedo, D.R., Hughes, R.M., Ligeiro, R., Ferreira, W.R., Castro, M.A., Junqueira, N.T., Oliveira, D.R., Firmiano, K.R., Kaufmann, P.R., Pompeu, P.S., 2014. The relative influence of catchment and site variables on fish and macroinvertebrate richness in cerrado biome streams. Landscape Ecology. 29, 1001-1016.
Macleod, C.K., Crawford, C.M., Moltschaniwskyj, N.A., 2004. Assessment of long term change in sediment condition after organic enrichment: defining recovery. Marine Pollution Bulletin. 49, 79-88.
Magurran, A.E., 2004. Measuring biological diversity. Blackwell Publishing.
Marchant, R., 1986. Some Quantitative Aspects of the Life History of Aquatic Insects in Temperate Astralian Rivers, Limnology in Australia. Springer, pp. 151-158.
Marchetti, M.P., Esteban, E., Smith, A.N., Pickard, D., Richards, A.B., Slusark, J., 2011. Measuring the ecological impact of long-term flow disturbance on the macroinvertebrate community in a large Mediterranean climate river. Journal of Freshwater Ecology. 26, 459-480.
MDFRC, 2009. Macroinvertebrate Survey. The Murray – Darling Freshwater Research Centre, Victoria, Australia.
Metcalfe, J.L., 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: History and present status in Europe. Environmental Pollution. 60, 101-139.
Metzeling, L., 1999. The impact of fish farm effluent on stream ecosystems-some results from EPA studies. Towards Best Practice in Land-based Salmonid Farming: Options for treatment, re-use and disposal of effluent, 60.
Metzeling, L., Chessman, B., Hardwick, R., Wong, V., 2003. Rapid assessment of rivers using macroinvertebrates: the role of experience, and comparisons with quantitative methods. Hydrobiologia. 510, 39-52.
Moore, J., 1979. Diversity and indicator species as measures of water pollution in a subarctic lake. Hydrobiologia. 66, 73-80.
Morgan, R.P., Jacobsen, R.E., Weisberg, S.B., McDowell, L.A., Wilson, H.T., 1991. Effects of flow alteration on benthic macroinvertebrate communities below the Brighton hydroelectric dam. Journal of freshwater ecology. 6, 419-429.
Munyika, S., Kongo, V., Kimwaga, R., 2014. River health assessment using macroinvertebrates and water quality parameters: A case of the Orange River in Namibia. Physics and Chemistry of the Earth, Parts A/B/C. 76-78, 140-148.
Mykrä, H., Heino, J., Muotka, T., 2007. Scale-related patterns in the spatial and environmental components of stream macroinvertebrate assemblage variation. Global Ecology and Biogeography. 16, 149-159.
Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature. 405, 1017-1024.
Nazar, A.A., Jayakumar, R., Tamilmani, G., 2013. Recirculating aquaculture systems.

Neori, A., Troell, M., Chopin, T., Yarish, C., Critchley, A., Buschmann, A.H., 2007. The need for a balanced ecosystem approach to blue revolution aquaculture. Environment: Science and Policy for Sustainable Development. 49, 36-43.
Nerbonne, B.A., Vondracek, B., 2001. Effects of local land use on physical habitat, benthic macroinvertebrates, and fish in the Whitewater River, Minnesota, USA. Environmental Management. 28, 87-99.
Nobre, A., Robertson-Andersson, D., Neori, A., Sankar, K., 2010. Ecological–economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture. 306, 116-126.
Norris, R., Lake, P., Swain, R., 1982. Ecological effects of mine effluents on the South Esk River, North-eastren Tasmania. III. Benthic Macroinvertebrates. Marine and Freshwater Research. 33, 789-809.
Ogbeibu, A., Oribhabor, B., 2002. Ecological impact of river impoundment using benthic macro-invertebrates as indicators. Water research. 36, 2427-2436.
Oliveira, S.V., Cortes, R., 2006. Environmental indicators of ecological integrity and their development for running waters in northern Portugal. Limnetica. 25, 479-498.
Omar, W.M.W., 2010. Perspectives on the use of algae as biological indicators for monitoring and protecting aquatic environments, with special reference to Malaysian freshwater ecosystems. Tropical life sciences research. 21, 51.
OME, 2012. An Algal Bioassessment Protocol for use in Ontario Rivers in: Environment, O.M.o.t. (Ed.). Queen's Printer for Ontario, Ontario, Canada.
Paisley, M., Walley, W.J., Trigg, D., 2011. Identification of macro-invertebrate taxa as indicators of nutrient enrichment in rivers. Ecological informatics. 6, 399-406.
Palmer, C., Maart, B., Palmer, A., O'Keeffe, J., 1996. An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. Hydrobiologia. 318, 153-164.
Parr, L.B., Mason, C.F., 2003. Long-term trends in water quality and their impact on macroinvertebrate assemblages in eutrophic lowland rivers. Water Research. 37, 2969-2979.
Parsons, M., Thoms, M., Norris, R., 2002. Australian river assessment system: AusRivAS physical assessment protocol. Monitoring river health initiative technical report.
Paul, M.J., Walsh, B., Oliver, J., Thomas, D., 2017. Algal Indicators in Streams: A Review of Their Application in Water Quality Management of Nutrient Pollution. United States Environmental Protection Agency.
Pearson, T., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16, 229-311.
Perry, S.A., Perry, W.B., 1986. Effects of experimental flow regulation on invertebrate drift and stranding in the Flathead and Kootenai Rivers, Montana, USA. Hydrobiologia. 134, 171-182.
Pillay, T., 2008. Aquaculture and the Environment. John Wiley & Sons.
Porter, S.D., Mueller, D.K., Spahr, N.E., Munn, M.D., Dubrovsky, N.M., 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biology. 53, 1036-1054.
Postel, S., Carpenter, S., 1997. Freshwater ecosystem services. Nature's Services: societal dependence on natural ecosystems, 195-214.
Prince, J., 1995. Limited effects of the sea urchin Echinometra mathaei (de Blainville) on the recruitment of benthic algae and macroinvertebrates into intertidal rock platforms at Rottnest Island, Western Australia. Journal of Experimental Marine Biology and Ecology. 186, 237-258.
Pulatsu, S., Rad, F., Koksal, G., Aydin, F., 2004a. The impact of rainbow trout farm effluents on water quality of Karasu stream, Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 4, 9-15.

Pulatsu, S., Rad, F., Köksal, G., Aydın, F., Benli, A.K., Topçu, A., 2004b. The impact of rainbow trout farm effluents on water quality of Karasu Stream, Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 4.

Quinn, J.M., Cooper, A.B., Davies-Colley, R.J., Rutherford, J.C., Williamson, R.B., 1997. Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill-country streams. New Zealand journal of marine and freshwater research. 31, 579-597.

Rak, A.-E., Said, I., Mohamed, M., 2011. EFFECTS OF LAND USE ON BENTHIC MACROINVERTEBRATE ASSEMBLAGES AT THREE RIVERS IN ENDAU CATCHMENT AREA, KLUANG, JOHOR, MALAYSIA. Journal of Applied Sciences in Environmental Sanitation. 6.

Rask, M., Nyberg, K., Markkanen, S.-L., Ojala, A., 1998. Forestry in catchments: effects on water quality, plankton, zoobenthos and fish in small lakes. Boreal Environment Research. 3, 75-86.

Reid, G.K., 2007. Chapter One: Nutrient Releases from Salmon Aquaculture.

Rennert, B., 1994. Water pollution by a land-based trout farm. Journal of applied ichthyology. 10, 373-378.

Rerat, A., Kaushik, S., 1995. Nutrition, animal production and the environment. Water Science and Technology. 31, 1-19.

Richards, C., HOST, G.E., ARTHUR, J.W., 1993. Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshwater Biology. 29, 285-294.

Richards, C., HARO, R., JOHNSON, L., HOST, G., 1997. Catchment and reach-scale properties as indicators of macroinvertebrate species traits. Freshwater Biology. 37, 219-230.

Ritz, D., Lewis, M., Shen, M., 1989. Response to organic enrichment of infaunal macrobenthic communities under salmonid seacages. Marine Biology. 103, 211-214.

Robinson, C.T., Uehlinger, U., Monaghan, M.T., 2003. Effects of a multi-year experimental flood regime on macroinvertebrates downstream of a reservoir. Aquatic Sciences. 65, 210-222.

Robinson, C.T., Aebischer, S., Uehlinger, U., 2004. Immediate and habitat-specific responses of macroinvertebrates to sequential, experimental floods. Journal of the North American Benthological Society. 23, 853-867.

Romero, K.C., Del Río, J.P., Villarreal, K.C., Anillo, J.C.C., Zarate, Z.P., Gutierrez, L.C., Franco, O.L., Valencia, J.W.A., 2017. Lentic water quality characterization using macroinvertebrates as bioindicators: An adapted BMWP index. Ecological indicators. 72, 53-66.

Rosenberg, D., Davies, I., Cobb, D., Wiens, A., 1998. Protocols for measuring biodiversity: Benthic macroinvertebrates in fresh waters. Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba.

Rosenberg, D.M., Resh, V.H., 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall.

Roset, N., Grenouillet, G., Goffaux, D., Pont, D., Kestemont, P., 2007. A review of existing fish assemblage indicators and methodologies. Fisheries Management and Ecology. 14, 393-405.

Ross, J., Macleod, C., 2006. Evaluation of Broadscale Environmental Monitoring Program (BEMP) data from. ratio. 10, 20.

Roy, A., Rosemond, A., Paul, M., Leigh, D., Wallace, J., 2003a. Stream macroinvertebrate response to catchment urbanisation (Georgia, USA). Freshwater biology. 48, 329-346.

Roy, A.H., Rosemond, A.D., Paul, M.J., Leigh, D.S., Wallace, J.B., 2003b. Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.). Freshwater Biology. 48, 329-346.

Ruiz-Zarzuela, I., Halaihel, N., Balcázar, J., Ortega, C., Vendrell, D., Pérez, T., Alonso, J., De Blas, I., 2009. Effect of fish farming on the water quality of rivers in northeast Spain. Water Science and Technology. 60, 663-671.

Scheltinga, D., Counihan, R., Moss, A., Cox, M., Bennett, J., 2004. User's guide for estuarine, coastal and marine indicators for regional NRM monitoring, 198 pp. Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management, Indooroopilly.

Schiemer, F., 2000. Fish as indicators for the assessment of the ecological integrity of large rivers. Springer.
Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K., Larsen, J.L., 2000. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Applied and environmental microbiology. 66, 4908-4915.
Schneider, S.C., Petrin, Z., 2017. Effects of flow regime on benthic algae and macroinvertebrates-A comparison between regulated and unregulated rivers. Science of the Total Environment. 579, 1059-1072.
Schultheis, A., Sanchez, M., Hendricks, A., 1997. Structural and functional responses of stream insects to copper pollution. Hydrobiologia. 346, 85-93.
Scrimgeour, G.J., Davidson, R.J., Davidson, J.M., 1988. Recovery of benthic macroinvertebrate and epilithic communities following a large flood, in an unstable, braided, New Zealand river. New Zealand journal of marine and freshwater research. 22, 337-344.
Selong, J.H., 1997. Impacts of aquaculture effluent on water quality and biotic communities in Virginia headwater streams. thesis. Virginia Tech.
Selong, J.H., Helfrich, L.A., 1998. Impacts of trout culture effluent on water quality and biotic communities in Virginia headwater streams. The Progressive Fish-Culturist. 60, 247-262.
Shackleton, M., Rees, G.N., 2016. DNA barcoding Australian macroinvertebrates for monitoring programs: benefits and current short comings. Marine and Freshwater Research. 67, 380-390.
Silva, C., Yáñez, E., Martín-Díaz, M.L., Riba, I., DelValls, T.A., 2013. Integrated ecotoxicological assessment of marine sediments affected by land-based marine fish farm effluents: physicochemical, acute toxicity and benthic community analyses. Ecotoxicology. 22, 996-1011.
Silvert, W., 1992. Assessing environmental impacts of finfish aquaculture in marine waters. Aquaculture. 107, 67-79.
Slepukhina, T., 1984. Comparison of different methods of water quality evaluation by means of oligochaetes, Aquatic Oligochaeta. Springer, pp. 183-186.
Slooff, W., 1983. Benthic macroinvertebrates and water quality assessment: Some toxicological considerations. Aquatic Toxicology. 4, 73-82.
Smal, H., Kornijow, R., Ligeza, S., 2005. The effect of catchment on water quality and eutrophication risk of five shallow lakes(Polesie region, Eastern Poland). Polish Journal of Ecology. 53, 313-327.
Smith, B.J., Davies, P.E., Munks, S.A., 2009. Changes in benthic macroinvertebrate communities in upper catchment streams across a gradient of catchment forest operation history. Forest Ecology and Management. 257, 2166-2174.
Smith, M.J., Kay, W.R., Edward, D.H.D., Papas, P.J., Richardson, K.S.J., Simpson, J.C., Pinder, A.M., Cale, D.J., Horwitz, P.H.J., Davis, J.A., Yung, F.H., Norris, R.H., Halse, S.A., 1999. AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. Freshwater Biology. 41, 269-282.
Smith, P., Donlon, J., Coyne, R., Cazabon, D.J., 1994. Fate of oxytetracycline in a fresh water fish farm: influence of effluent treatment systems. Aquaculture. 120, 319-325.
Sponseller, R., Benfield, E., Valett, H., 2001a. Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshwater Biology. 46, 1409-1424.
Sponseller, R.A., Benfield, E.F., Valett, H.M., 2001b. Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshwater Biology. 46, 1409-1424.
Spruzen, F., Richardson, A.M., Woehler, E., 2008. Spatial variation of intertidal macroinvertebrates and environmental variables in Robbins Passage wetlands, NW Tasmania. Hydrobiologia. 598, 325-342.
Stark, J.D., Group, N.Z.M.W., 2001. Protocols for sampling macroinvertebrates in wadeable streams. Cawthron Institute.

Stephens, W.W., Farris, J.L., 2004. Instream community assessment of aquaculture effluents. Aquaculture. 231, 149-162.
Stevenson, R.J., Smol, J.P., 2003. Use of algae in environmental assessments. Academic Press, San Diego, CA, pp. 775-804.
Storey, A.W., Edward, D.H.D., Gazey, P., 1991. Surber and kick sampling: a comparison for the assessment of macroinvertebrate community structure in streams of south-western Australia. Hydrobiologia. 211, 111-121.
Szmant, A., Forrester, A., 1996. Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. Coral Reefs. 15, 21-41.
Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture. 226, 181-189.
Taseli, B., 2009. Influence of land-based fish farm effluents on the water quality of Yanýklar Creek. Int. J. Environ. Res. 3, 45-56.
TEER, 2011. Tamar Estuary 2011 Report Card: Ecosystem Health Assessment Program

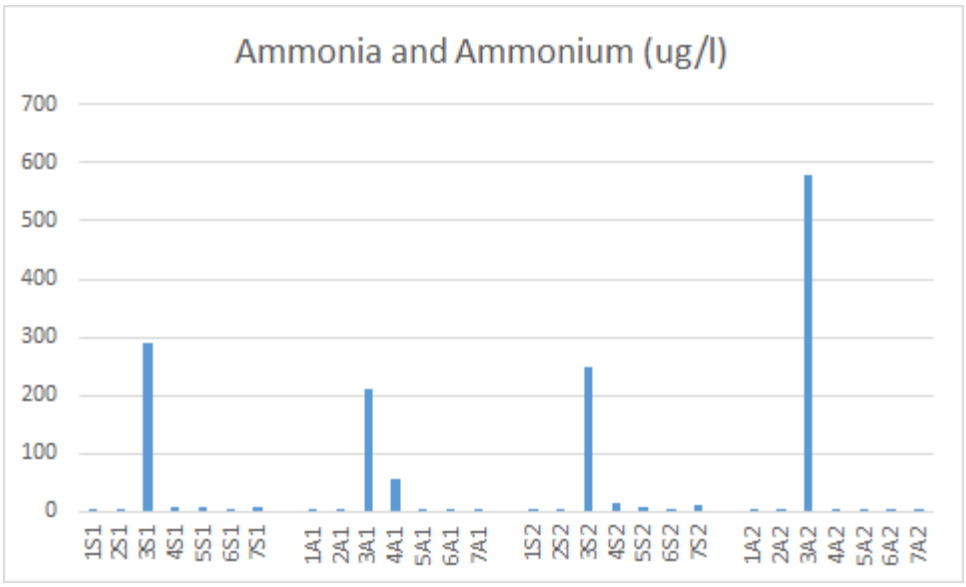
the Tamar Estuary and Esk Rivers (TEER) Ecosystem Health

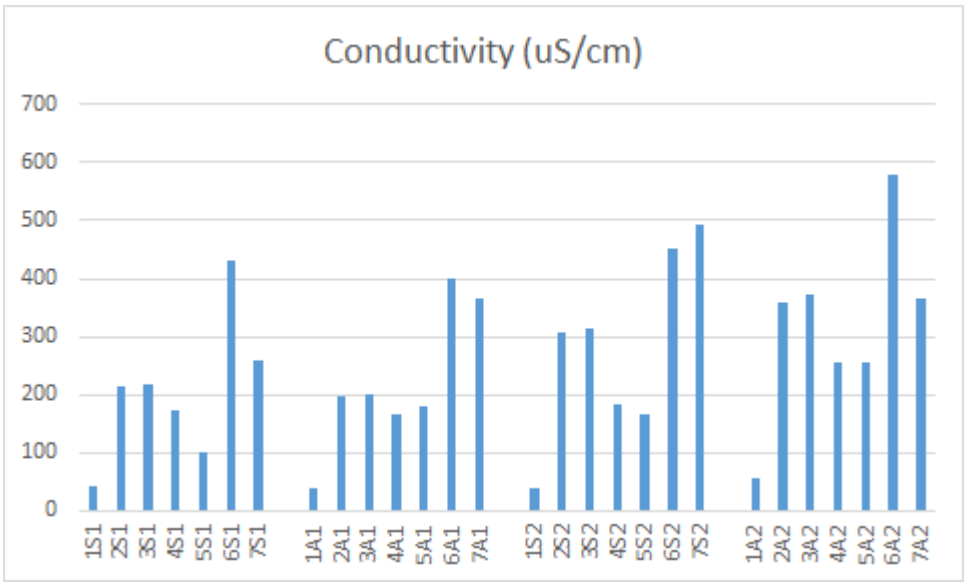
Assessment Program (EHAP).
Tello, A., Corner, R., Telfer, T.C., 2010. How do land-based salmonid farms affect stream ecology? Environmental Pollution. 158, 1147-1158.
Tong, S.T., Chen, W., 2002. Modeling the relationship between land use and surface water quality. Journal of environmental management. 66, 377-393.
Troell, M., Halling, C., Nilsson, A., Buschmann, A.H., Kautsky, N., Kautsky, L., 1997. Integrated marine cultivation of Gracilaria chilensis (Gracilariales, Rhodophyta) and salmon cages for reduced environmental impact and increased economic output. Aquaculture. 156, 45-61.
TSGA, 2019. History: Salmon's success story. Tasmanian Salmonid Growers Association, Hobart, Tasmania.
Turcios, A., Papenbrock, J., 2014. Sustainable treatment of aquaculture effluents—what can we learn from the past for the future? Sustainability. 6, 836-856.
Usseglio-Polatera, P., Beisel, J.N., 2002. Longitudinal changes in macroinvertebrate assemblages in the Meuse River: anthropogenic effects versus natural change. River Research and Applications. 18, 197-211.
Uzunov, J., Košel, V., Sládeček, V., 1988. Indicator value of freshwater Oligochaeta. Acta Hydrochimica et hydrobiologica. 16, 173-186.
Van Griensven, A., Meixner, T., Grunwald, S., Bishop, T., Diluzio, M., Srinivasan, R., 2006. A global sensitivity analysis tool for the parameters of multi-variable catchment models. Journal of hydrology. 324, 10-23.
Varnosfaderany, M.N., Ebrahimi, E., Mirghaffary, N., Safyanian, A., 2010. Biological assessment of the Zayandeh Rud River, Iran, using benthic macroinvertebrates. Limnologica - Ecology and Management of Inland Waters. 40, 226-232.
Walker, T., 2014. Aquaculture Status Report and Industry Resources, Austasia Aquaculture magazine. Turtle Press Pty Ltd, Tasmania, Australia.
Wang, X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquaculture Environment Interactions. 2, 267-283.
Webb, J.A., 2012a. Effects of trout farms on stream macroinvertebrates: linking farm-scale disturbance to ecological impact. Aquaculture Environment Interactions. 3, 23-32.
Webb, J.A., 2012b. Effects of trout farms on stream macroinvertebrates: linking farm-scale disturbance to ecological impact. Aquaculture Environment Interactions. 3, 23-32.
Weijters, M., Janse, J., Alkemade, R., Verhoeven, J., 2009. Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems. 19, 104-112.

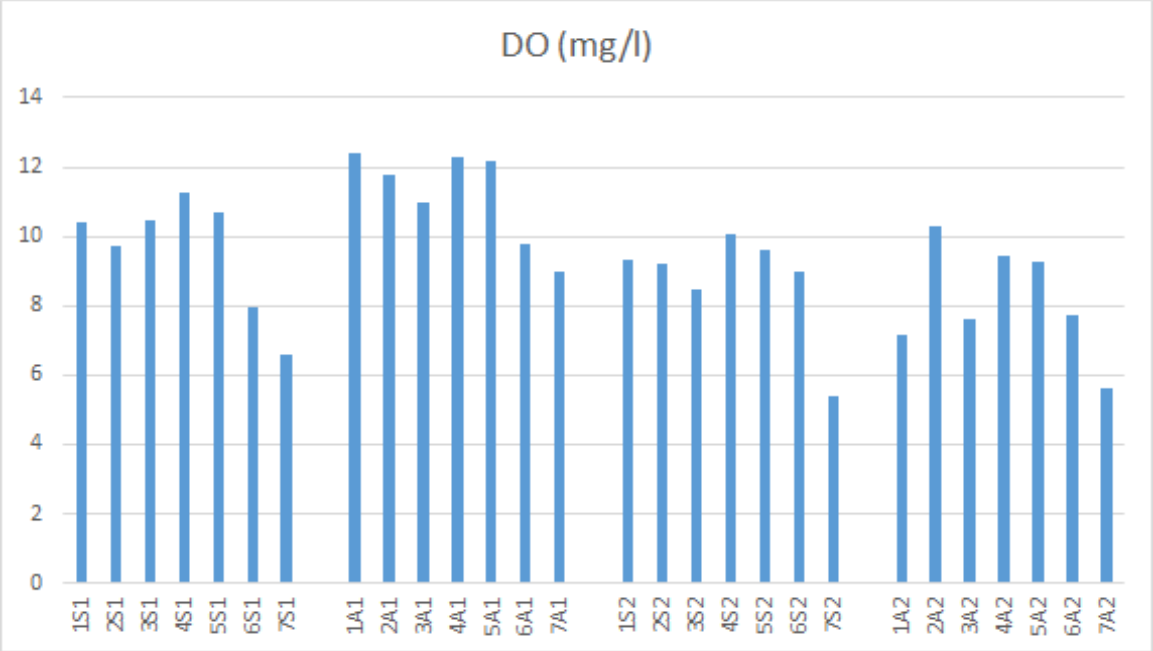
Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. The journal of geology. 30, 377-392.
Whiles, M.R., Brock, B.L., Franzen, A.C., Dinsmore, S.C., 2000. Stream invertebrate communities, water quality, and land-use patterns in an agricultural drainage basin of northeastern Nebraska, USA. Environmental Management. 26, 563-576.
Whitehurst, I.T., 1991. TheGammarus: Asellus ratio as an index of organic pollution. Water Research. 25, 333-339.
Whitehurst, I.T., Lindsey, B.I., 1990. The impact of organic enrichment on the benthic macroinvertebrate communities of a lowland river. Water Research. 24, 625-630.
Williams, W.D., 1980. Australian freshwater life: the invertebrates of Australian inland waters. Macmillan Education AU.
Winberg, P.C., Lynch, T.P., Murray, A., Jones, A.R., Davis, A.R., 2007. The importance of spatial scale for the conservation of tidal flat macrobenthos: An example from New South Wales, Australia. Biological Conservation. 134, 310-320.
Wood, P., Armitage, P., 1999. Sediment deposition in a small lowland stream—management implications. Regulated Rivers: Research & Management: An International Journal Devoted to River Research and Management. 15, 199-210.
Wright, J., Berrie, A., 1987. Ecological effects of groundwater pumping and a natural drought on the upper reaches of a chalk stream. River Research and Applications. 1, 145-160.
Wright, R., 1991. RAIN Project. Report for the years 1988, 1989 and 1990.
Wu, R.S.S., 1995. The environmental impact of marine fish culture: Towards a sustainable future. Marine Pollution Bulletin. 31, 159-166.
Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: A case study in the sub-tropical environment. Marine Environmental Research. 38, 115-145.
Xu, H., Paerl, H.W., Qin, B., Zhu, G., Gaoa, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography. 55, 420-432.
Ye, L.-X., Ritz, D., Fenton, G., Lewis, M., 1991. Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. Journal of Experimental Marine Biology and Ecology. 145, 161-174.
Zalidis, G., Stamatiadis, S., Takavakoglou, V., Eskridge, K., Misopolinos, N., 2002. Impacts of agricultural practices on soil and water quality in the Mediterranean region and proposed assessment methodology. Agriculture, Ecosystems & Environment. 88, 137-146.
Živić, I., Marković, Z., Filipović-Rojka, Z., Živić, M., 2009. Influence of a trout farm on water quality and macrozoobenthos communities of the receiving stream (Trešnjica River, Serbia). International Review of Hydrobiology. 94, 673-687.

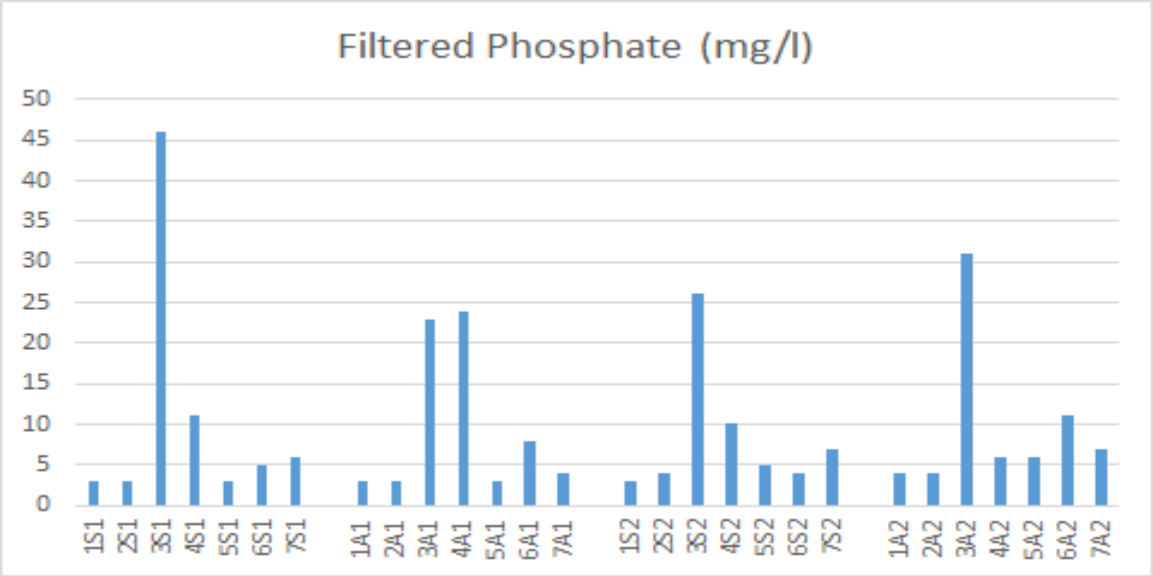
Appendices

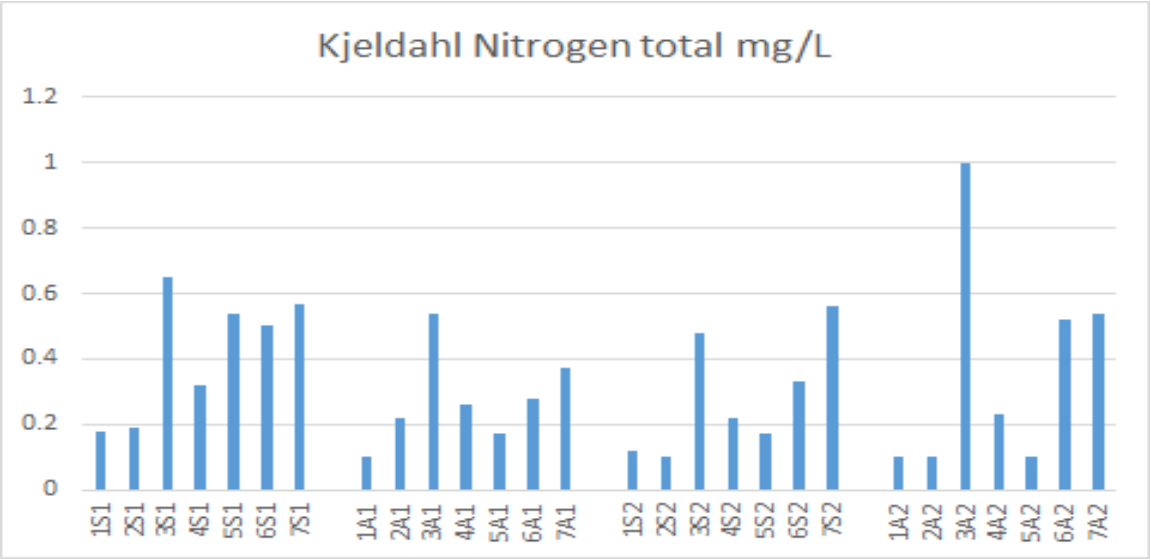
Appendix 1: Water quality parameters between 7 sites in the Derwent Catchment over 4 sampling time (Chapter 5) (Site codes: 1: Broad, 2: Florentine 1, 3: Florentine 2, 4: Tyenna End, 5: Styx, 6: Dee, 7: Ouse) (Season codes: S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017)

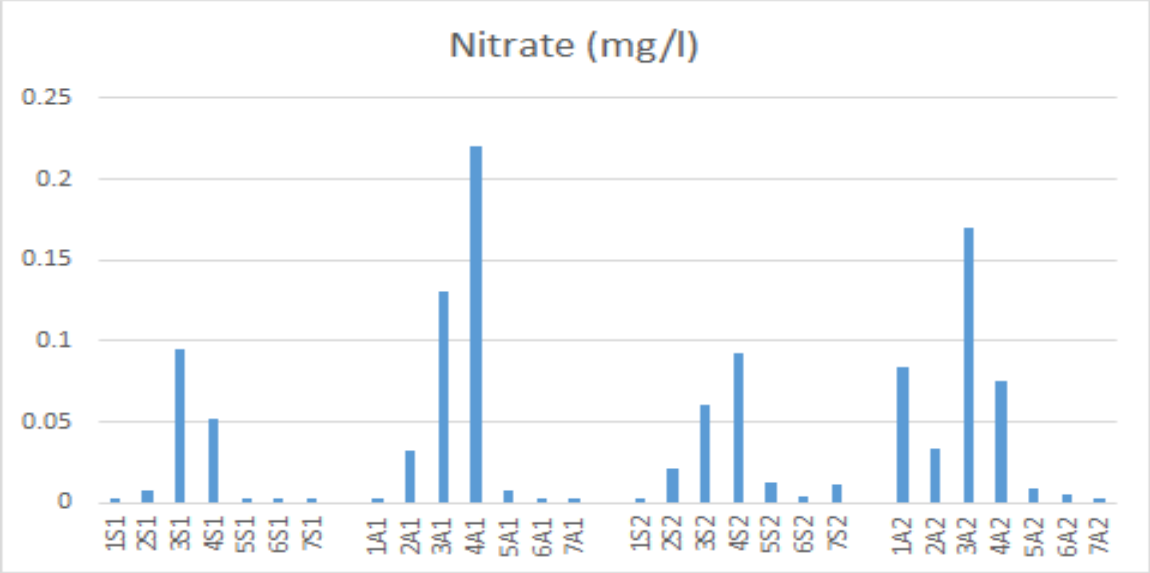


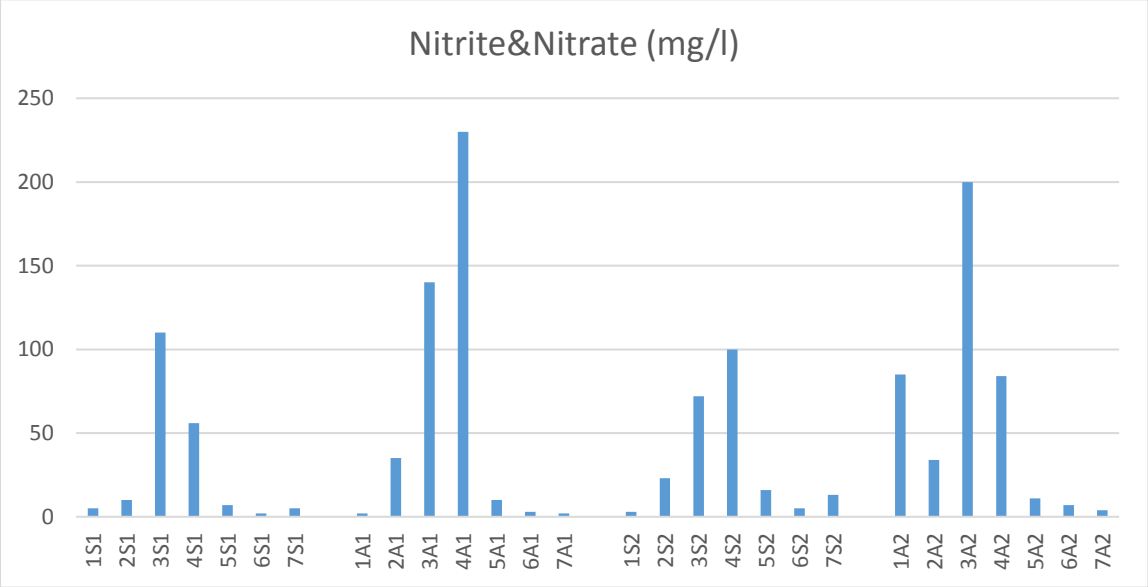


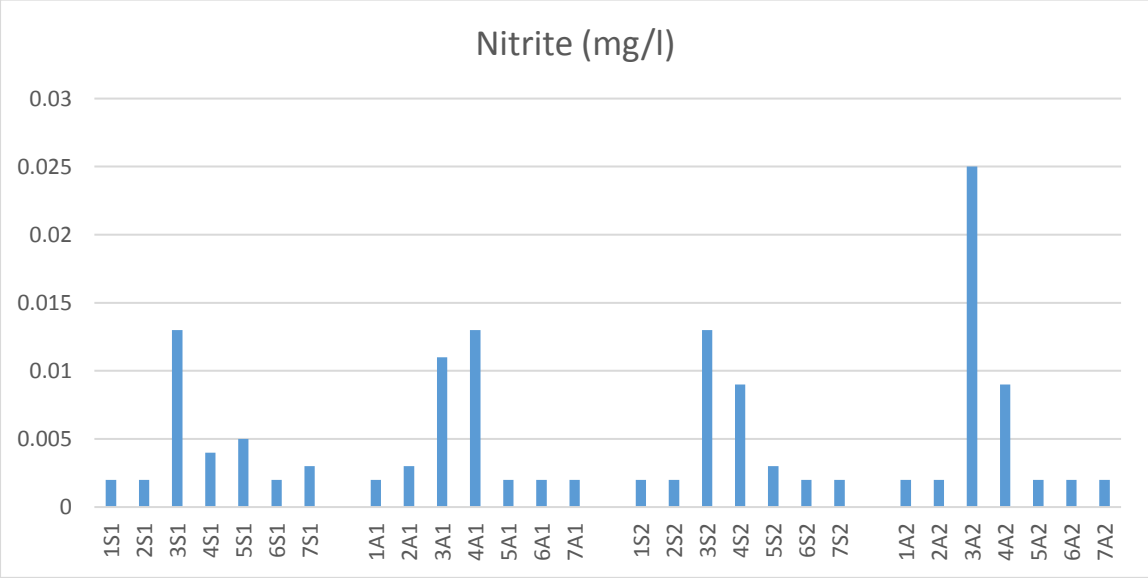

DO (mg/l)
14
12
10
8
6
4
2
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

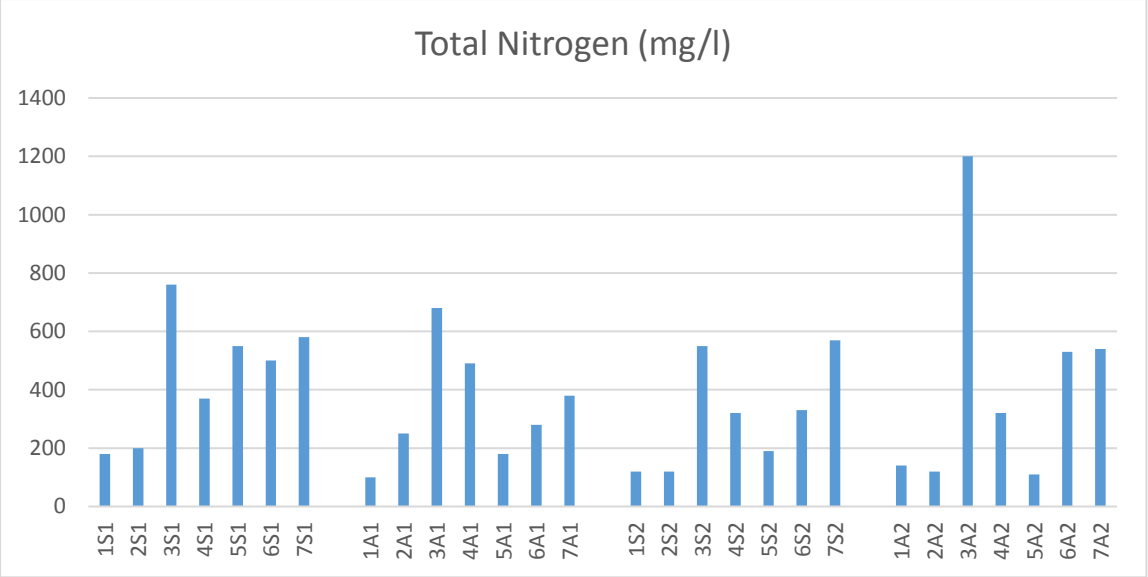

Filtered Phosphate (mg/l)
50
45
40
35
30
25
20
15
10
5
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

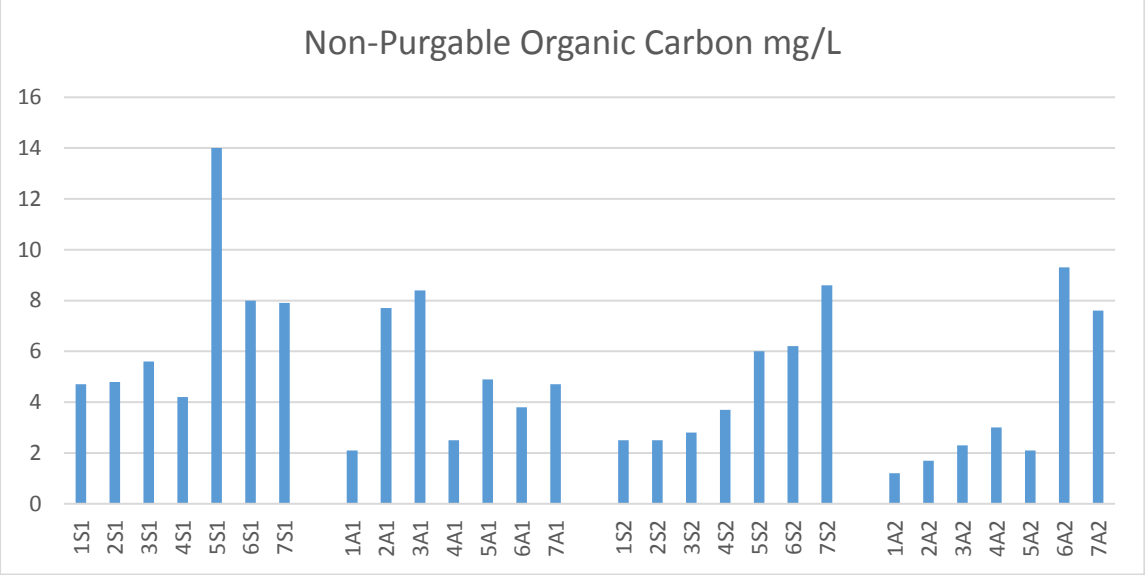

Kjeldahl Nitrogen total mg/L
1.2
1
0.8
0.6
0.4
0.2
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

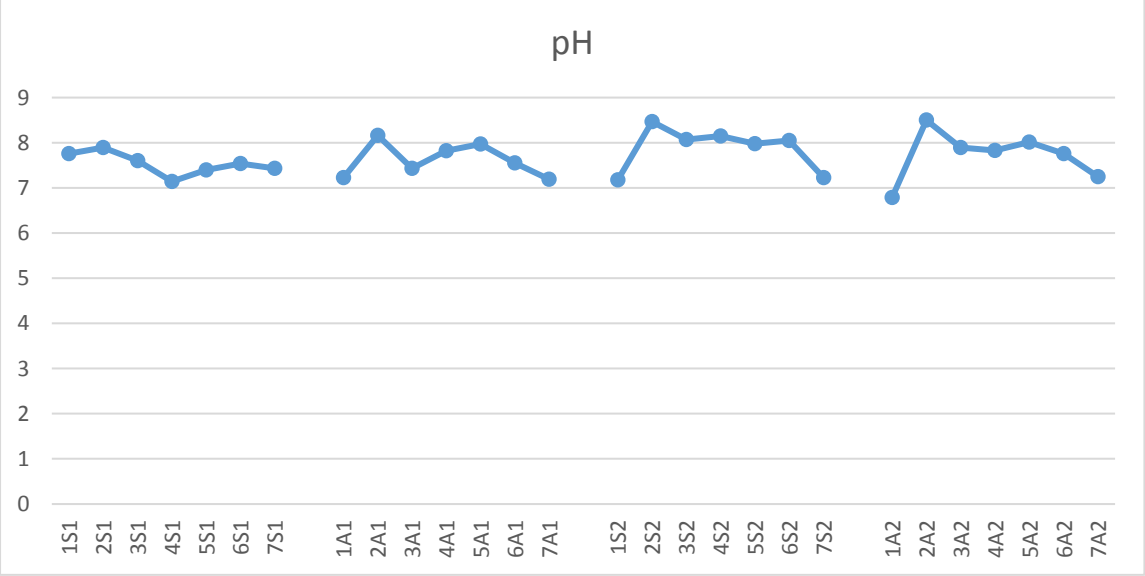

Nitrate (mg/l)
0.25
0.2
0.15
0.1
0.05
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

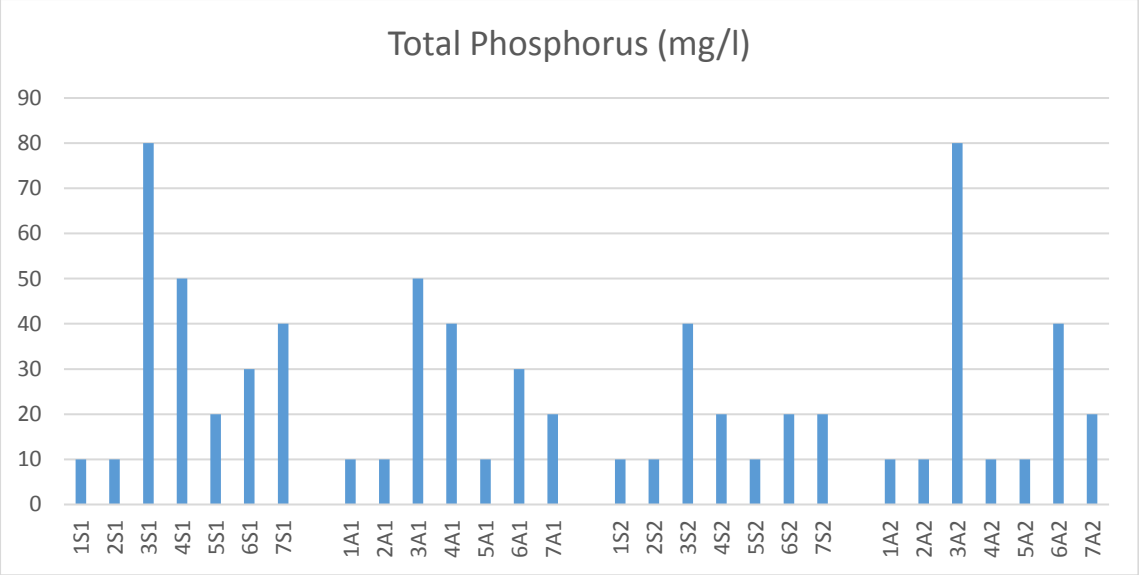

Nitrite&Nitrate (mg/l)
250
200
150
100
50
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

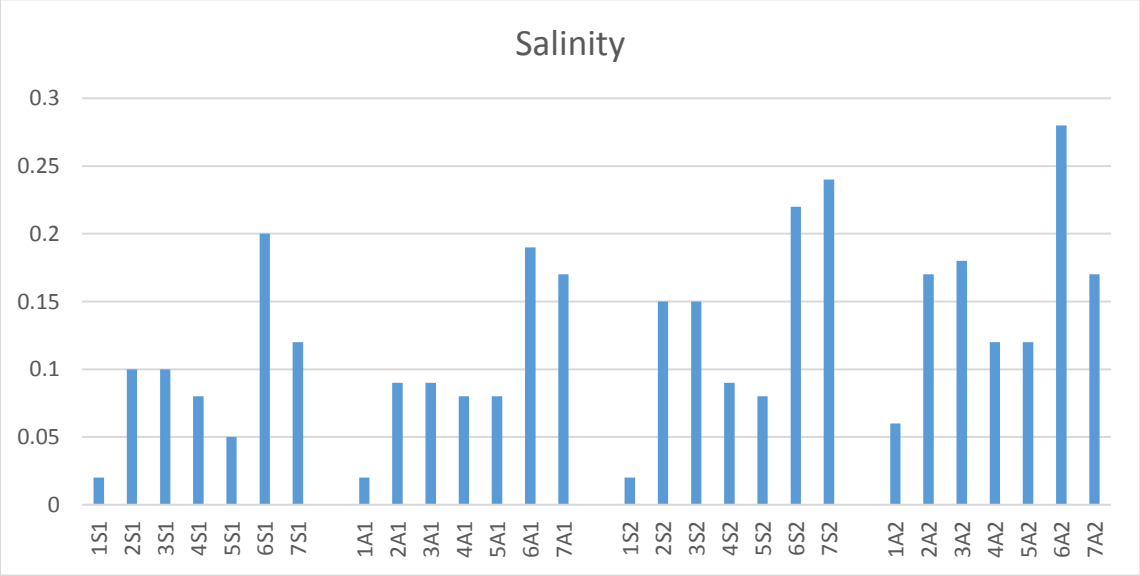

Nitrite (mg/l)
0.03
0.025
0.02
0.015
0.01
0.005
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

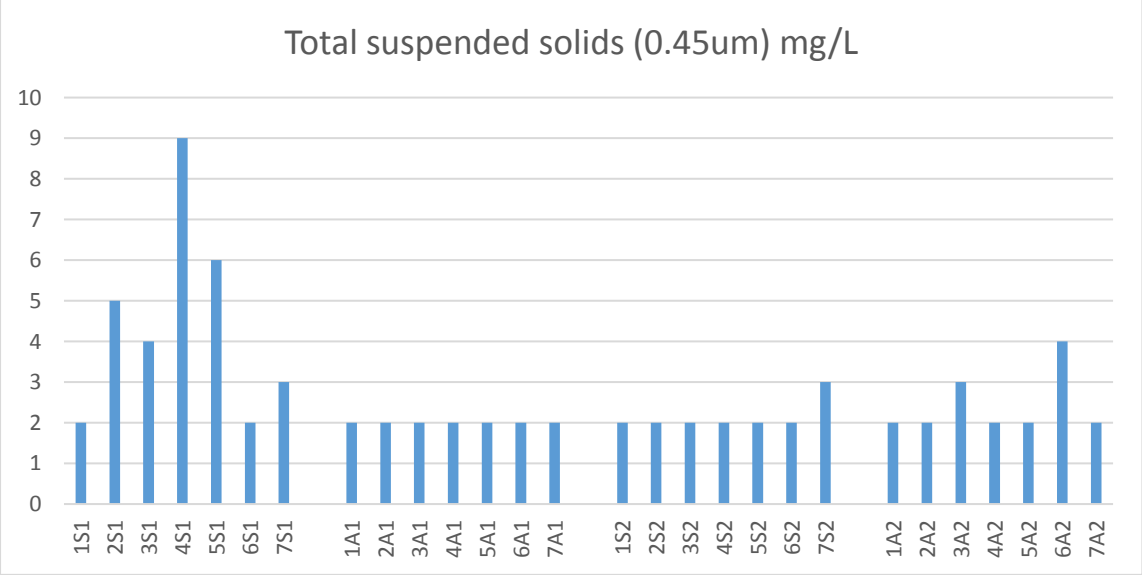

Total Nitrogen (mg/l)
1400
1200
1000
800
600
400
200
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

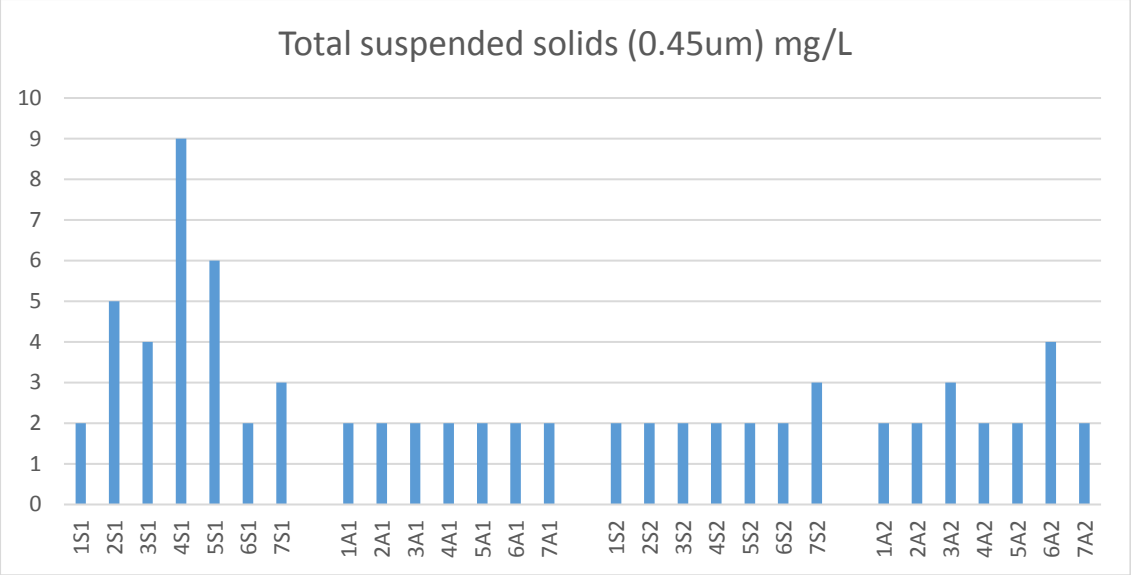

Non-Purgable Organic Carbon mg/L
16
14
12
10
8
6
4
2
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

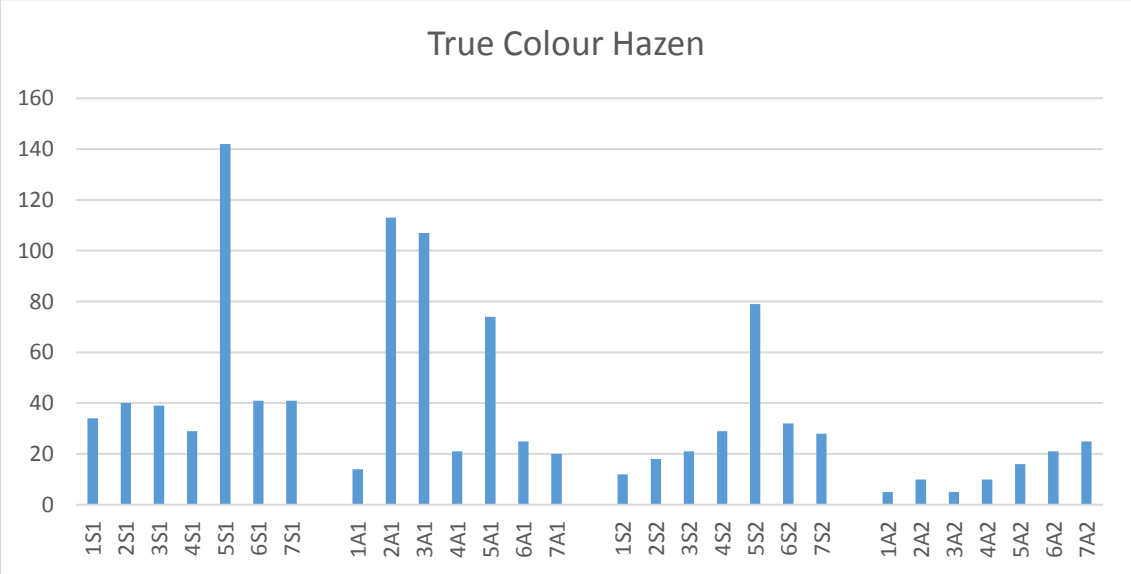

pH
9
8
7
6
5
4
3
2
1
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

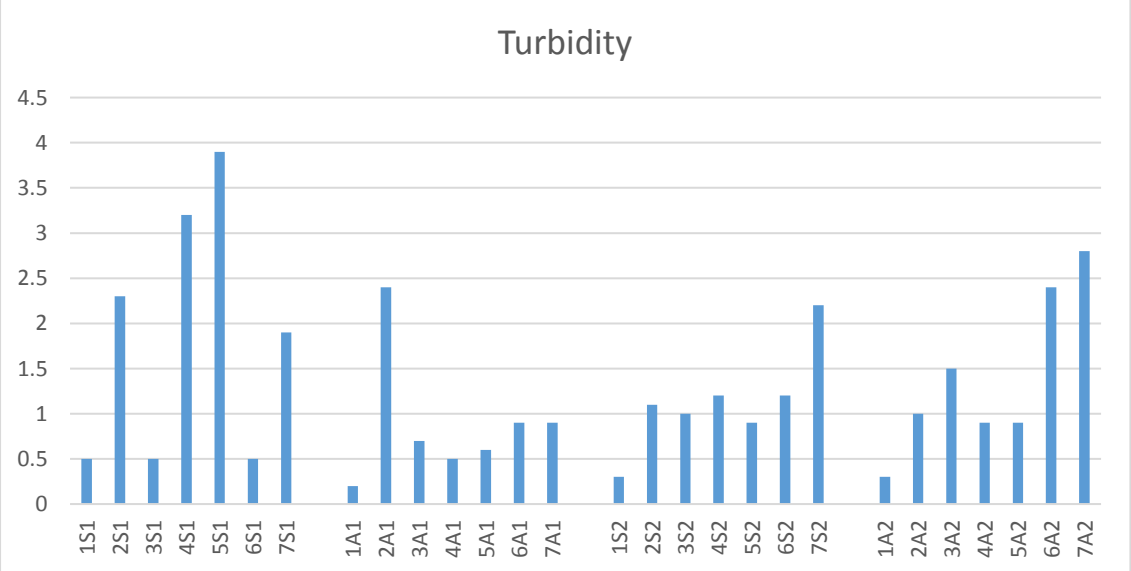

Total Phosphorus (mg/l)
90
80
70
60
50
40
30
20
10
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

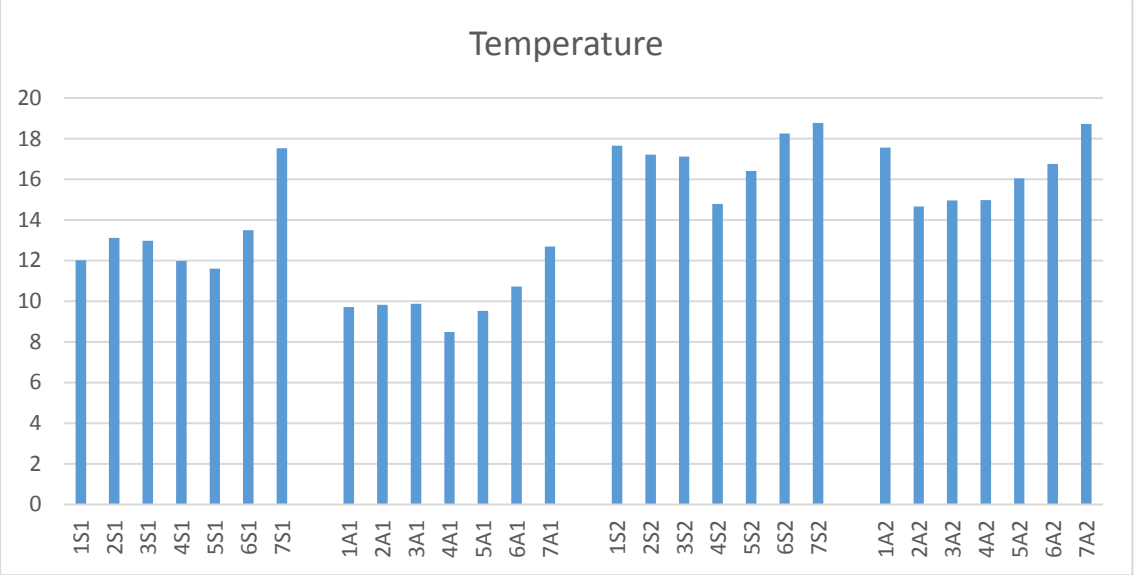

Salinity
0.3
0.25
0.2
0.15
0.1
0.05
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

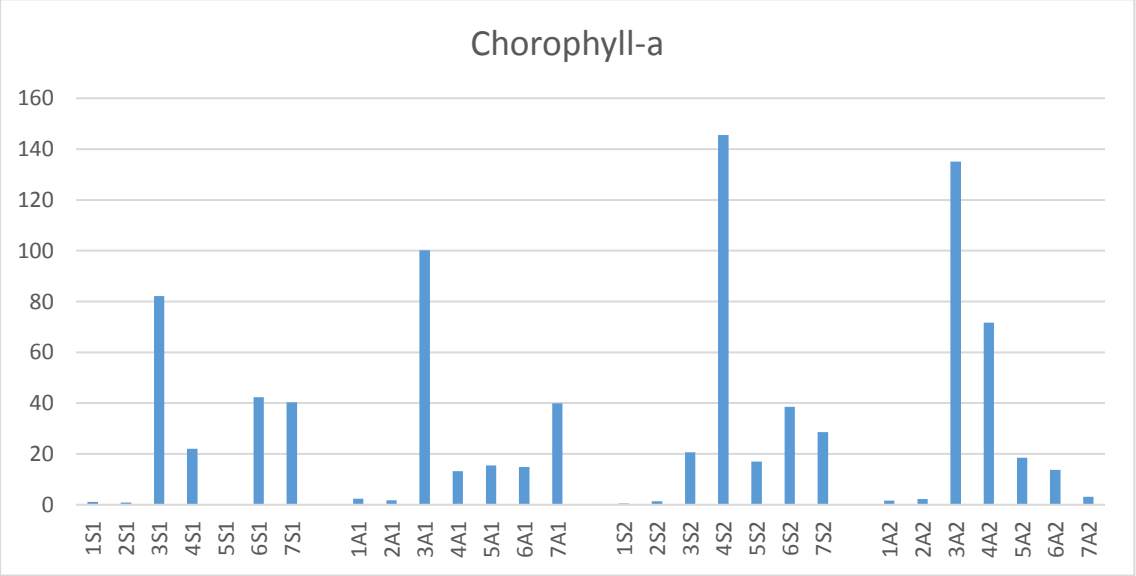

Total suspended solids (0.45um) mg/L
10
9
8
7
6
5
4
3
2
1
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2


Total suspended solids (0.45um) mg/L
10
9
8
7
6
5
4
3
2
1
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

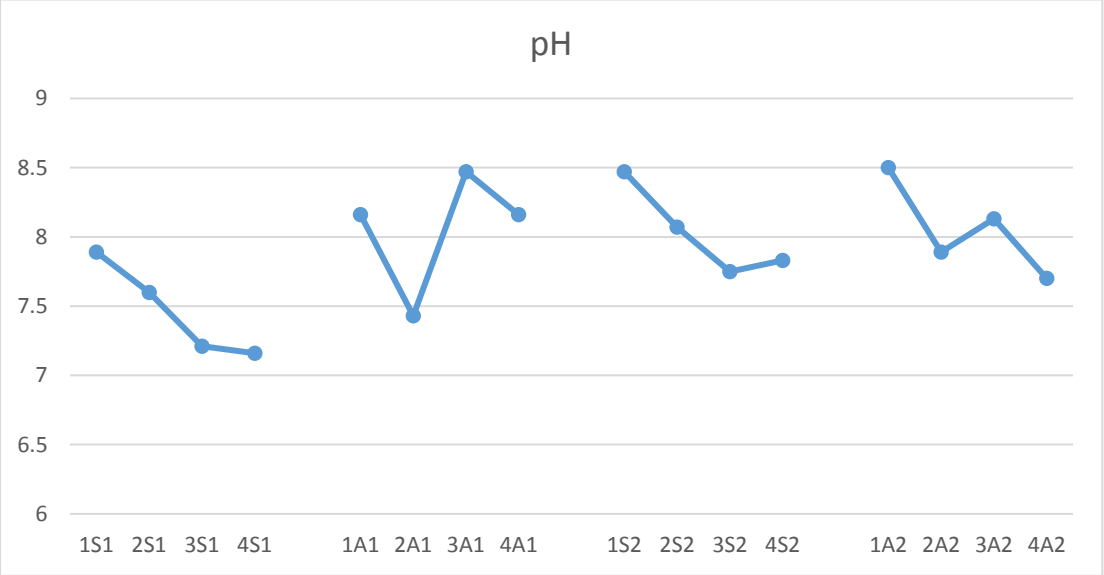

True Colour Hazen
160
140
120
100
80
60
40
20
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

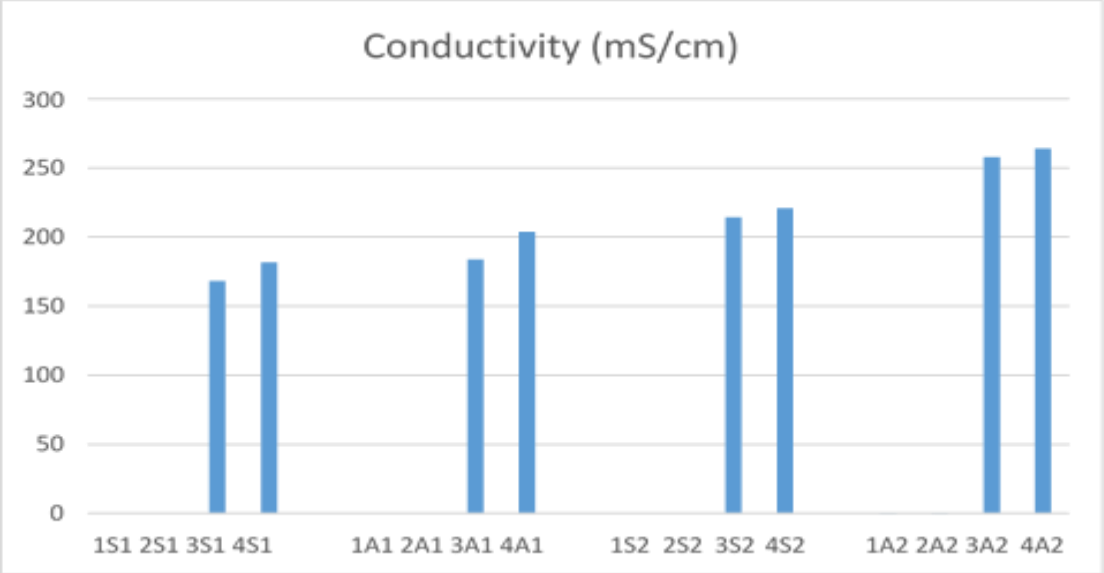

Turbidity
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1S1 2S1 3S1 4S1 5S1 6S1 7S1 1A1 2A1 3A1 4A1 5A1 6A1 7A1 1S2 2S2 3S2 4S2 5S2 6S2 7S2 1A2 2A2 3A2 4A2 5A2 6A2 7A2

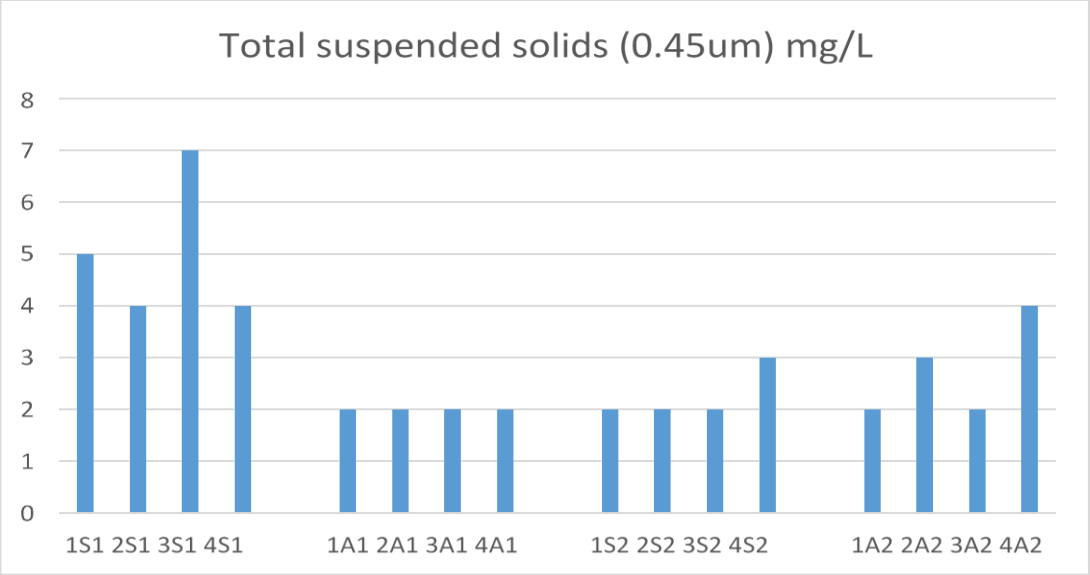

Temperature
20
18
16
14
12
10
8
6
4
2
0
1S1
2S1
3S1
4S1
5S1
6S1
7S1
1A1
2A1
3A1
4A1
5A1
6A1
7A1
1S2
2S2
3S2
4S2
5S2
6S2
7S2
1A2
2A2
3A2
4A2
5A2
6A2
7A2

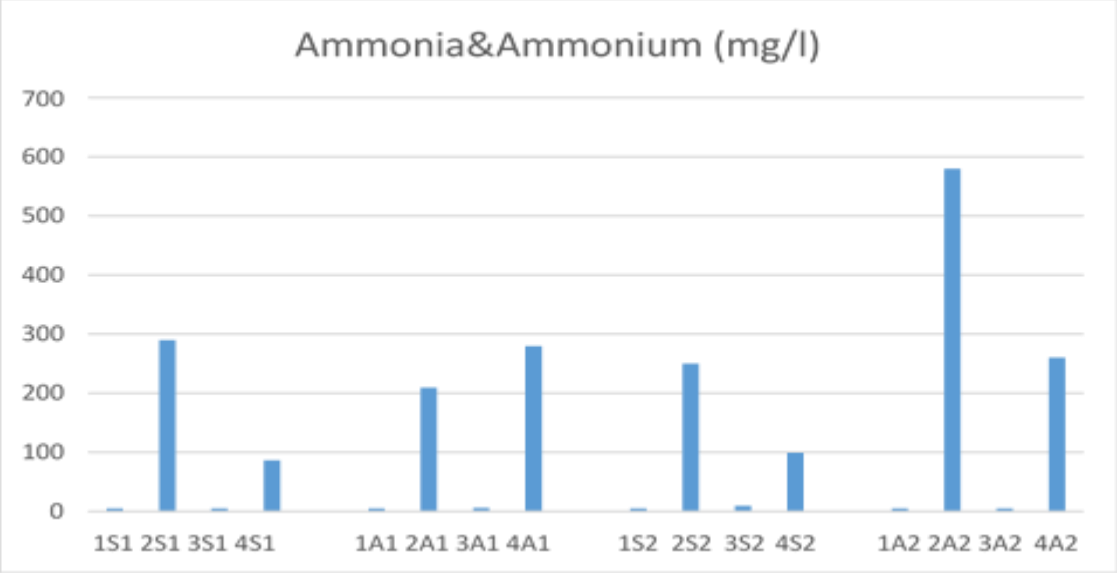

Chorophyll-a
160
140
120
100
80
60
40
20
0
1S1
2S1
3S1
4S1
5S1
6S1
7S1
1A1
2A1
3A1
4A1
5A1
6A1
7A1
1S2
2S2
3S2
4S2
5S2
6S2
7S2
1A2
2A2
3A2
4A2
5A2
6A2
7A2

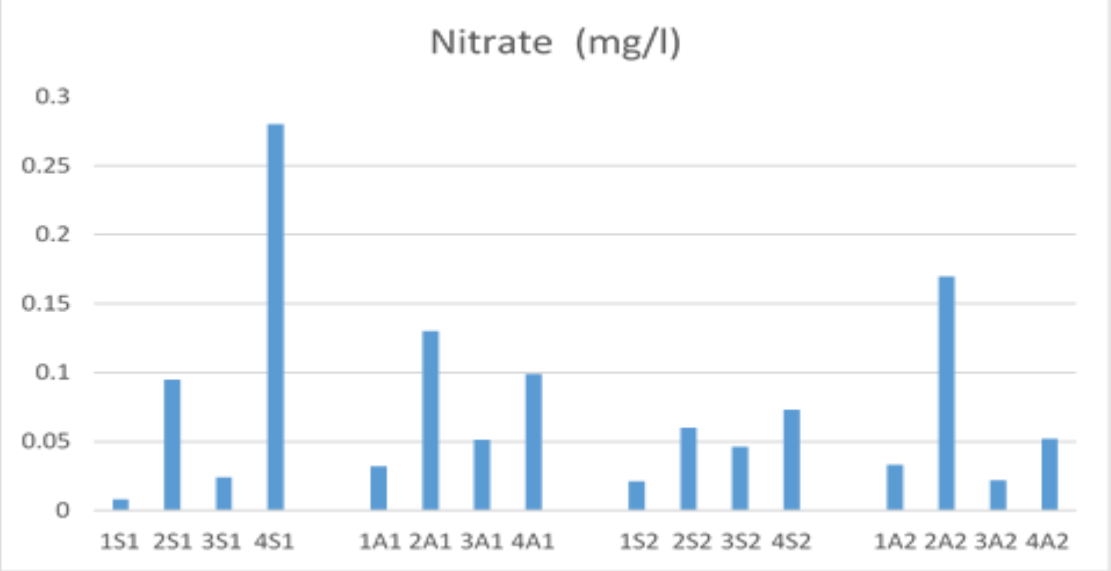
Appendix 2: Water quality parameters between upstream and downstream stations in two aquaculture sites (Chapter 5) (Site codes: 1: Florentine upstream, 2: Florentine downstream, 3: Russell Falls upstream, 4: Russell Falls downstream) (Season codes: S1: summer 2016, A1: autumn 2016, S2: summer 2017, A2: autumn 2017)

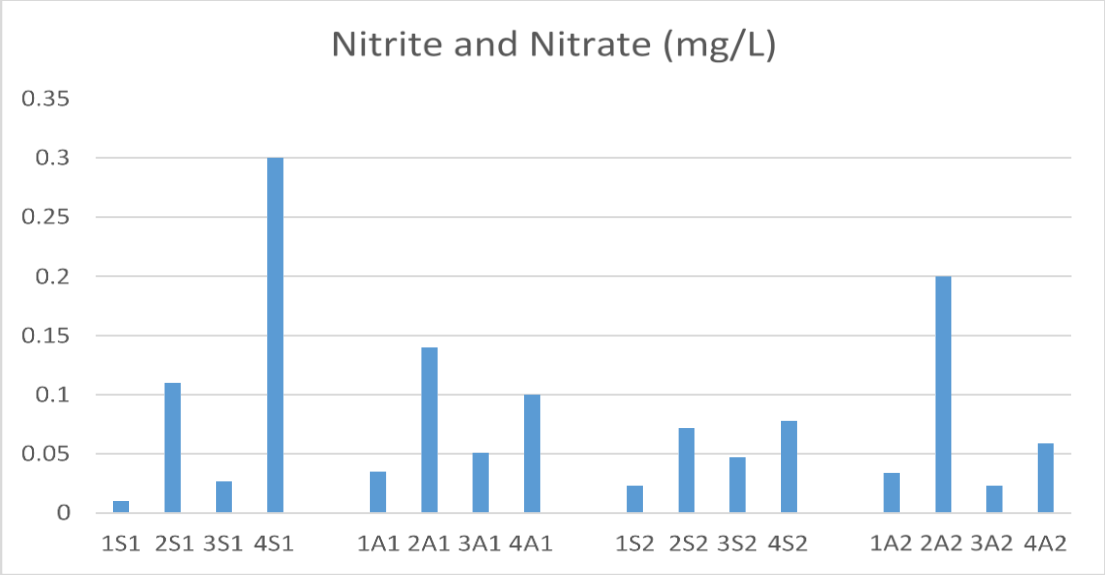


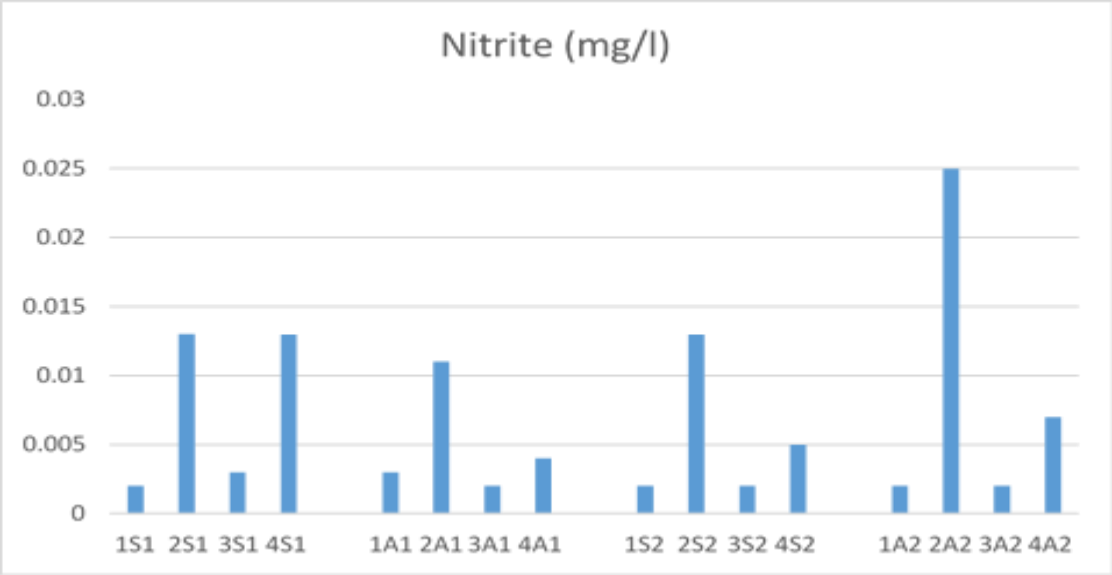


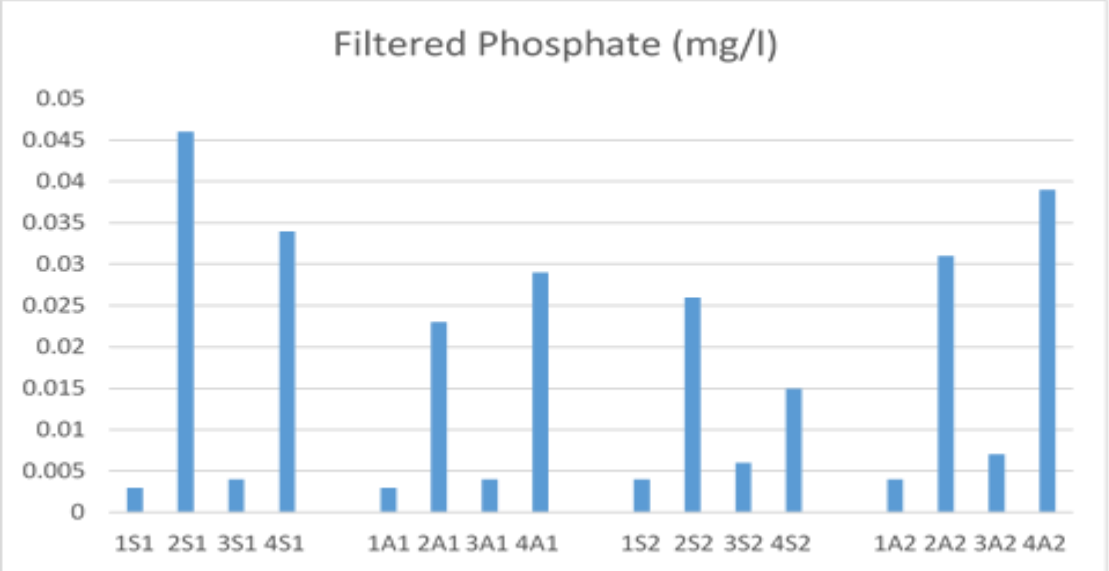

Total suspended solids (0.45um) mg/L
8
7
6
5
4
3
2
1
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2

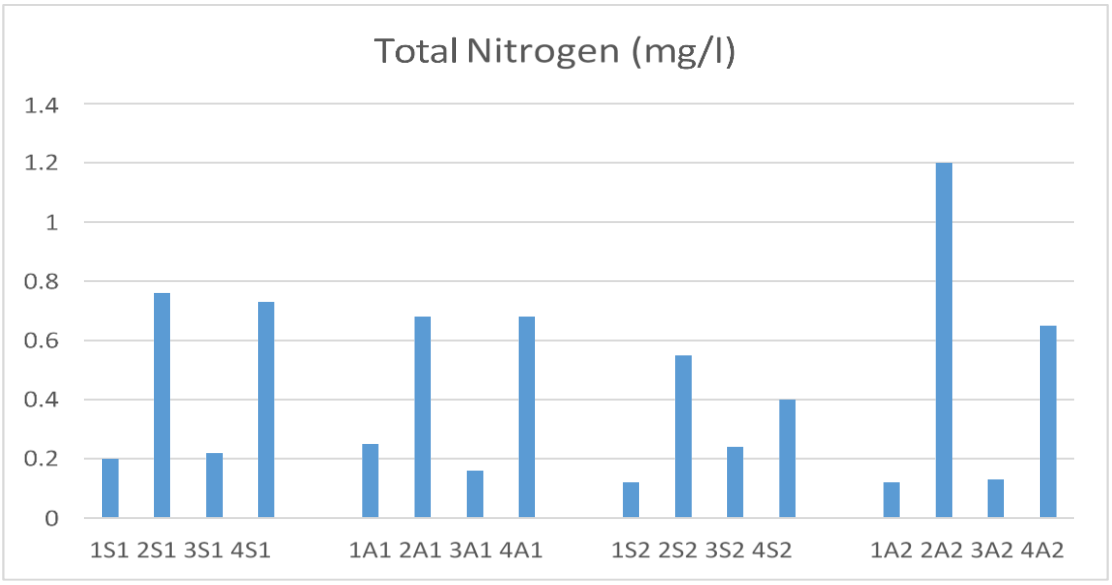

Ammonia&Ammonium (mg/l)
700
600
500
400
300
200
100
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2

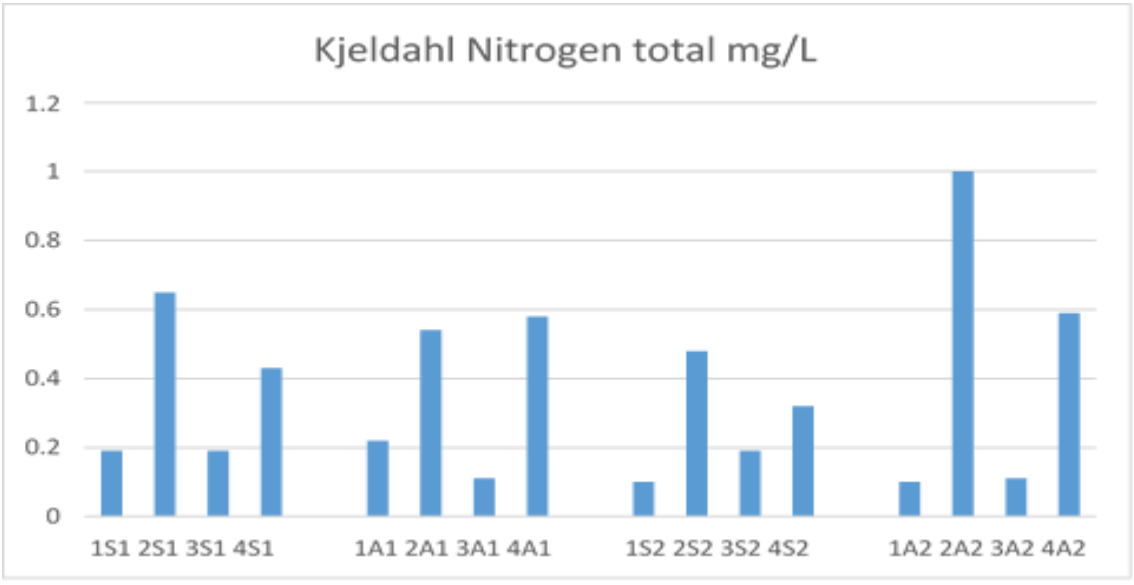

Nitrate (mg/l)
0.3
0.25
0.2
0.15
0.1
0.05
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2

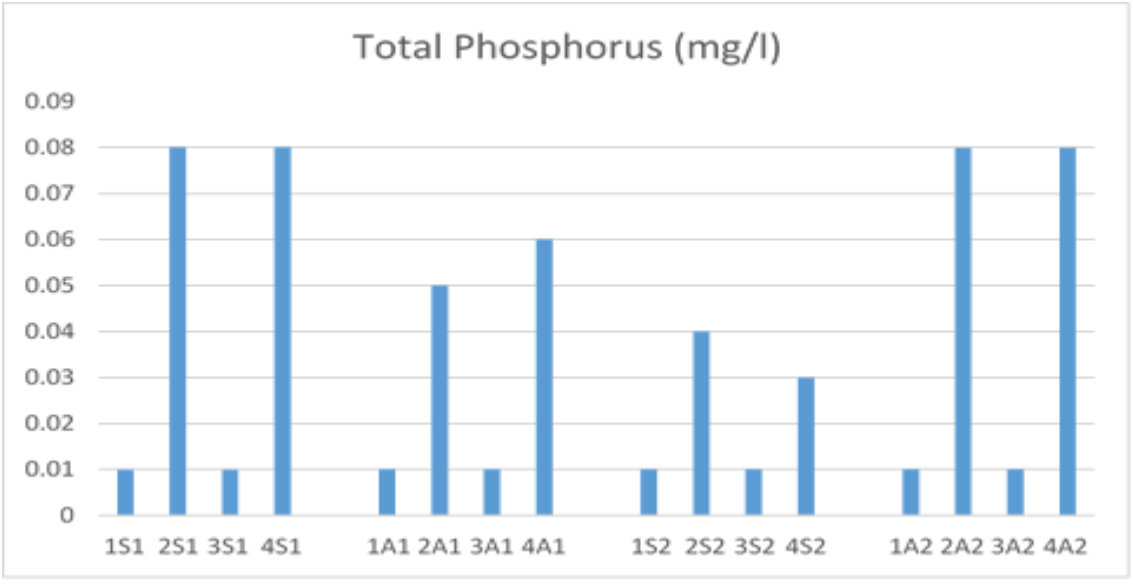

Nitrite and Nitrate (mg/L)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1S1 2S1 3S1 4S1 1A1 2A1 3A1 4A1 1S2 2S2 3S2 4S2 1A2 2A2 3A2 4A2

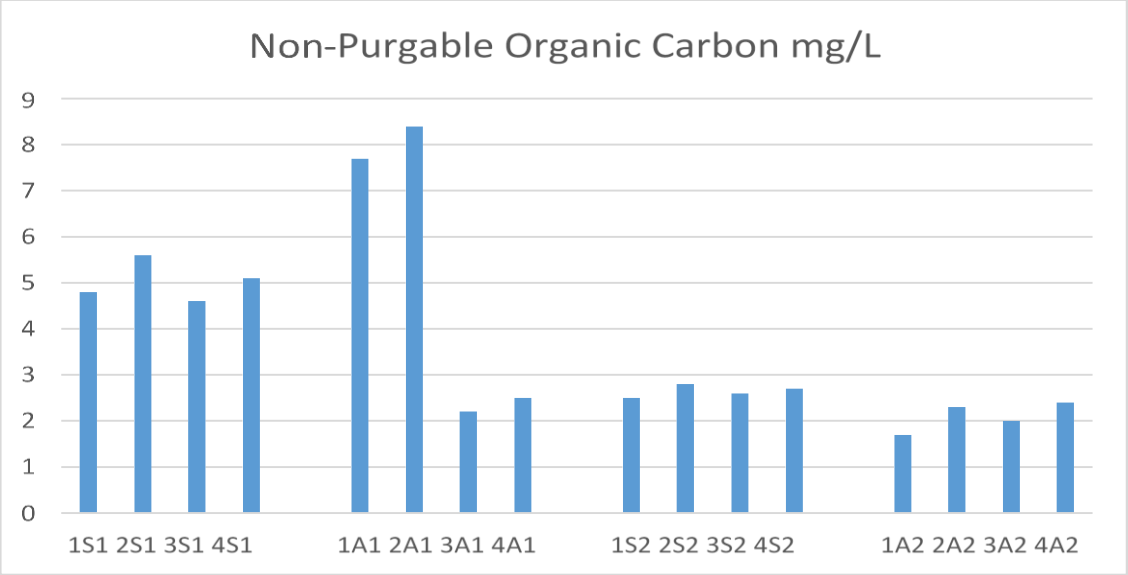

Nitrite (mg/l)
0.03
0.025
0.02
0.015
0.01
0.005
0
1S1 2S1 3S1 4S1 1A1 2A1 3A1 4A1 1S2 2S2 3S2 4S2 1A2 2A2 3A2 4A2

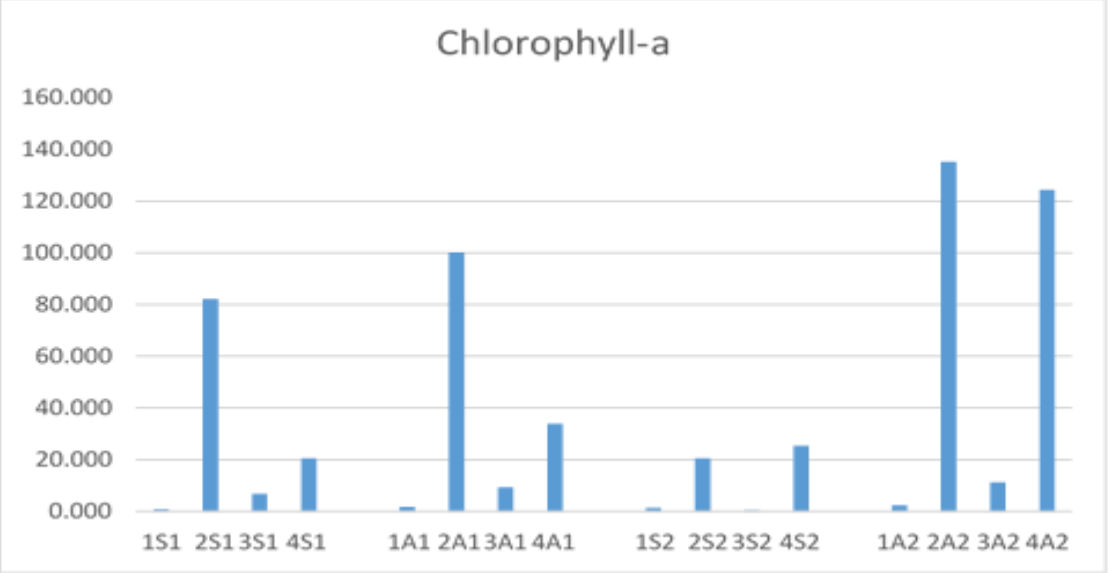

Filtered Phosphate (mg/l)
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1S1 2S1 3S1 4S1 1A1 2A1 3A1 4A1 1S2 2S2 3S2 4S2 1A2 2A2 3A2 4A2


Total Nitrogen (mg/l)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2

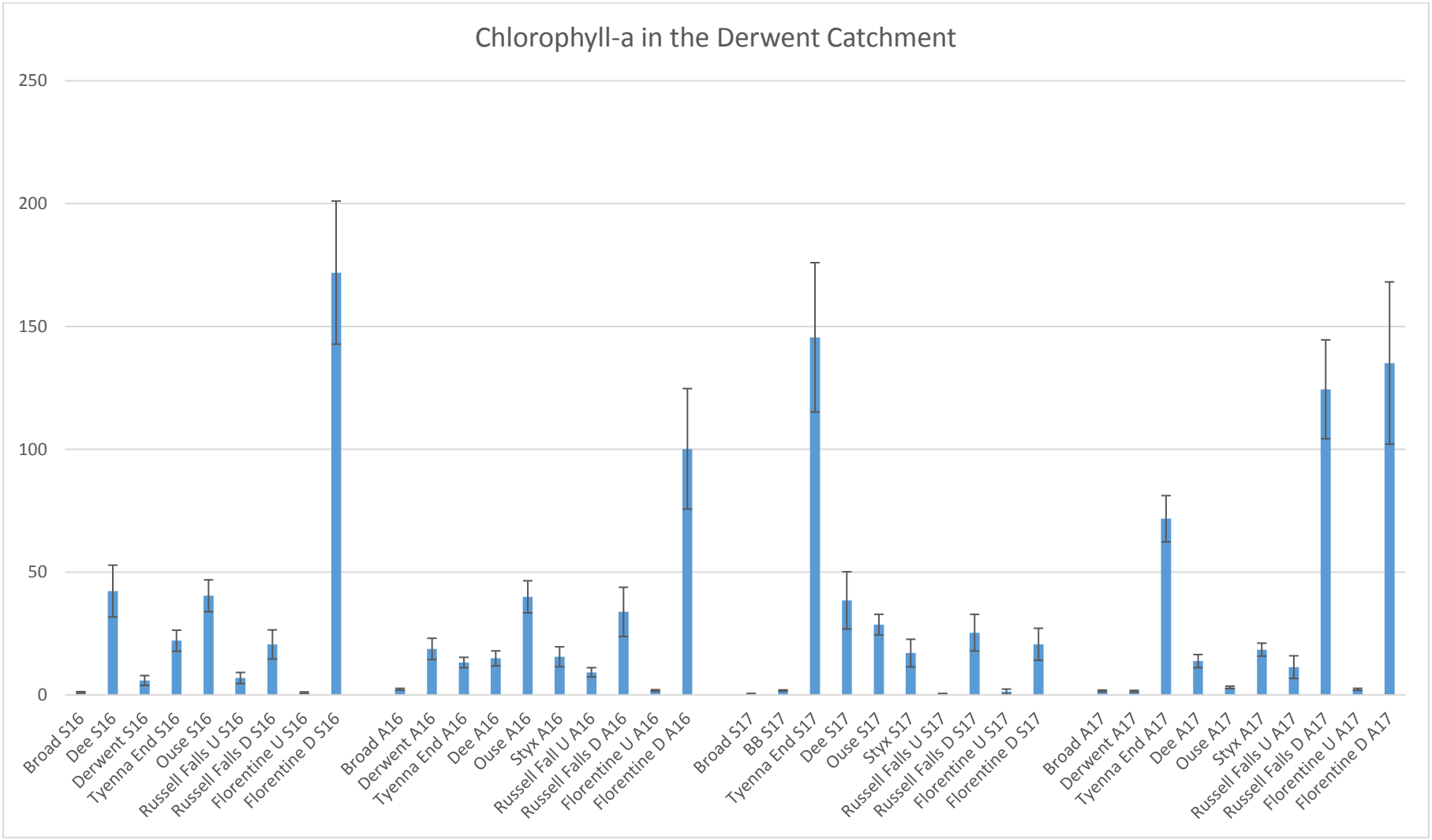

Kjeldahl Nitrogen total mg/L
1.2
1
0.8
0.6
0.4
0.2
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2


Total Phosphorus (mg/l)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2


Non-Purgable Organic Carbon mg/L
9
8
7
6
5
4
3
2
1
0
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2


Chlorophyll-a
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
1S1 2S1 3S1 4S1
1A1 2A1 3A1 4A1
1S2 2S2 3S2 4S2
1A2 2A2 3A2 4A2

Appendix 3: Chlorophyll-a between ten sites at the Derwent Catchment over four sampling times



Appendix 4: Background of farm tonnage and strategies at two hatcheries at Florentine and Brumbys Creek

The four salmon hatcheries monitored in this study have since the time of sampling expanded their use of recirculating aquaculture systems (RAS) and other strategies (e.g. feeding) to reduce effluent released to settlement ponds and ultimately to the rivers. At the time of this study all farms displayed different production levels and strategies and are summarised as follows.

The hatchery on the Florentine in conjunction with another neighbouring hatchery at Wayatinah on the River Derwent is operated by Saltas Pty Ltd. The Florentine site produced c. 240 tonne Atlantic salmon on-growing approximately 2-2.6 million fry (sourced from Wayatinah) to smolt (range 100-150 g; average 120-130g) across the year focusing specifically on photo-manipulated production of out-of-season smolts and marine pre-smolts. Out-of-season smolt (c. 40% total) were grown on site between November and March while marine pre-smolt (c. 60% total) were produced between April and October. The farm employs both RAS and flow-through tank systems (all photo-controlled); fish at 10-100g are grown in the RAS while fish are on-grown to smolt in the flow through systems. Solid and nutrient wastes are removed via RAS management and via the settlement pond before effluent water exits through the outfall into the Florentine River.

Petuna operates the hatchery on Brumbys Ck in northern Tasmania, combining RAS tank technology with flow through tanks and raceway systems to produce c.450 tonne fish at the time of this study. Two RAS systems support egg/fry production and smolt production. In 2016-17 the site annually grew c. 2.5 million Atlantic salmon smolt (average 150g), 0.3 million two year old rainbow trout fingerlings (average 300g) and c. 10-15,000 broodstock (5,000 x 5 kg and 5-10,000 x <2 kg developing brood). Fish from 50g were on-grown in both indoor RAS and outdoor flow through systems at a ratio of 50:50%. Rainbow trout were transferred to sea in March-April and September while salmon were transferred to sea as out-of-season, marine pre-smolt and spring smolt between April and October. The FCR achieved was 1.1:1 and approximately 20t/week (1000 t/year) of solid waste was removed via the solids filtration of the RAS systems before water exited the farm via a settlement pond.